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THE TREATMENT OF DIABETES MELLITUS WITH INSOLUBLE INSULIN COMPOUNDS[1]

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In 1935 Bischoff and Maxwell (1) reported that tannic acid was an effective precipitant of hormones of protein character and that the resulting salt was biologically more effective than the uncombined hormone. When commercial insulin was combined with tannic acid a salt formed which was absorbed more slowly and had a more prolonged effect upon the blood sugar of experimental animals than did the insulin alone. The effect was so striking that the question of its application to the treatment of diabetes in human patients immediately arose. To date 13 patients have been treated, under controlled conditions, with insulin tannate either alone or in combination with commercial insulin. Sporadic observations are available on 5 additional patients.

INSULIN TANNATE

Since the chemical constitution of tannic acid is still controversial, since more than one compound is present, and since precipitates of insulin tannate form a colloid in low electrolyte concentration, the question of the amount of tannic acid to be mixed with the insulin for clinical trial presented many difficulties. The material used in this study was prepared in the Chemical Laboratory of the Santa Barbara Cottage Hospital by Dr. Fritz Bischoff, to whom I am deeply indebted for the privilege of trying insulin tannate clinically. His analyses based on the nitrogen determinations of insulin and insulin tannate precipitated from a 1 per cent solution of sodium chloride at a pH acid to the isoelectric point, indicated that 4 parts of tannic acid were combined with 6 parts of insulin. Three milligrams of tannic acid would therefore be the correct amount, theoretically, to use with 100 μ of insulin. Since commercial insulin is not so pure as crystalline insulin, the amount of tannic acid actually present was less than the theoretical amount predicted from the nitrogen calculation. However, some free tannic acid may still have been present in the material actually used, because the amount of tannic acid which was theoretically necessary for complete precipitation was not present, because of an equilibrium reaction.

[1] Read before the Southern California Medical Association April 3, 1936 at Santa Barbara, California.

Tannic acid is supplied in sterile, rubber capped vials. The solution has a clear russet color. Equal volumes of the solution are mixed in the hypodermic syringe with those of commercial insulin of U-100 strength. The resulting mixture is slightly cloudy and opalescent. It contains 3 mg. of tannic acid per 100 μ of insulin and has approximately the same pH as the commercial insulin. Upon standing 24 hours the insulin tannate precipitates. Because the stability of this mixture is under question the 2 solutions are mixed immediately before injection. Since the addition of tannic acid delays the hypoglycemic reaction, biologic standardization of insulin tannate will probably be difficult (2).

Technique of Injection. For the injection of insulin tannate the usual hypodermic syringe and needle may be used. The syringe should be sterile, dry, and cold. The rubber caps of the 2 vials (one containing the tannic acid and the other the commercial insulin) should be cleansed with iodine and alcohol. Extra precaution in the sterility of the technique seems to be necessary because no preservative has been added to the tannic acid solution. Insulin is withdrawn into the syringe first and the tannic acid second. The two are thoroughly mixed in the syringe before injection. If an opalescent mixture is not obtained the mixing is considered inadequate. A deeper injection is made than is the case usually with commercial insulin. The area where the injection has been made is massaged for several seconds.

Local Reactions. Since the insulin tannate preparation used probably contains some free tannic acid, the condition of the skin and subcutaneous tissues about the area of injection become a problem for concern. To date we have given well over 200 injections. Local reactions have been encountered where the solution has not been strictly sterile and in persons who frequently suffer from local reactions to commercial insulin. Early in our experience a superficial erythematous lesion was occasionally encountered but culture of the mixture injected showed that contamination with a mould-like organism had occurred. More sterile technique has eliminated this lesion. Occasionally the patients complain of more 'stinging' during the injection than with commercial insulin. In some patients tender subcutaneous lumps have remained at the site of injection for 24 to 48 hours. In most patients no visible or palpable change at the site of injection could be detected 12 hours after the injection, at the time of discharge from the hospital, no skin blotches or subcutaneous lumps being present on either the patient (case H. V.) who received the maximum number of injections (51) or on the patient (case F. V.) who received the maximum amount of tannic acid (27.2 mg.).

Our experience and that of Bischoff and Maxwell (1) with laboratory animals, suggests that the subcutaneous injection of tannic acid in the amounts used in this study is without detriment to the skin. The amount which we have injected, of course, falls far short of that which is applied locally in the treatment of burns, where from 2 to 6 ounces of 5 per cent tannic acid (3 to 9 gm.) are used. In our opinion the skin reactions occasionally encountered with insulin tannate are not a significant drawback to its use.

OBSERVATIONS

Our observations on the therapeutic use of insulin tannate in the treatment of human diabetes mellitus may be divided into 2 sections: (a) those in which insulin tannate was used alone to control the diabetes and its effect compared to that of commercial insulin, and (b) those in which the action of the insulin tannate was compared to that of protamine insulinate (3, 4)[2].

[2] The protamine insulinate used in this work has been generously supplied by Eli Lilly and Company, Indianapolis, Indiana.

Only those observations made under controlled conditions will be cited in detail.

Clinical Effect of Insulin Tannate. To date 11 patients who required from 2 to 4 doses of commercial insulin daily have received for varying periods of time insulin tannate alone. In all 11 patients it has been found to be an adequate substitute for commercial insulin, capable of controlling their diabetes. Five patients required fewer injections per day of insulin tannate than of commercial insulin to maintain the same degree of control; the remainder required the same number. In 9 of the 11 cases, insulin tannate proved to be more effective than commercial insulin, as shown by either a reduced require-

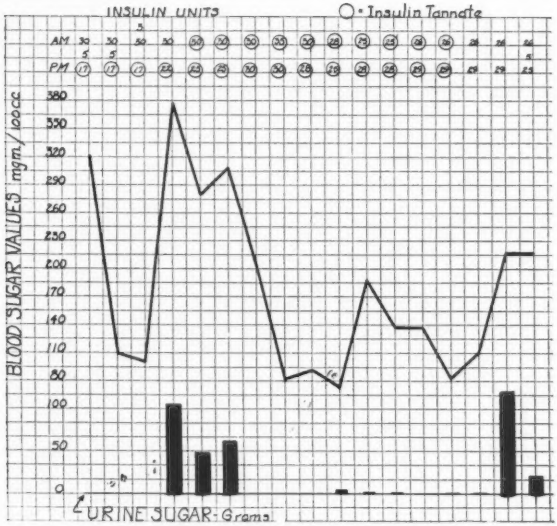

Chart 1. Case 1 (J. M.). Note decrease in blood and urinary sugar when insuline tannate was used.

ment in actual units, or by a lower blood sugar level, or both. The prolonged action of insulin tannate is apparent from the effect on the fasting blood sugar the morning following the injection of the salt at night. This effect is particularly striking in the first 2 cases to be described. When a reduced unitage of insulin tannate controlled the diabetes, the reduction was about 20 to 25 per cent less than the total amount of commercial insulin necessary.

Case 1. J. M., a well developed boy, aged 18 years, had had diabetes for 7 years. Prior to the experiment he had been hospitalized for 1 month. Glycosuria and hyperglycemia had never been adequately controlled. His daily insulin dose varied from 44 to 55 u., divided into 4 doses. During the experiment his diet was C-192, P-92, F-96, Cal. 2202. He ate all of his food each day and exercised at the same hour and to about the same extent. His body weight varied from 70.1 to 71.1 kg. Beginning June 15, 1935 his diabetes was controlled by giving him 55 u. of insulin as follows: 30 u. of commercial insulin before breakfast, 5 u. before lunch, and 17 u. of insulin tannate before supper. Glycosuria disappeared and the fasting blood sugar fell to normal. Then he was given 2 doses of insulin tannate daily and

as Chart 1 shows, his diabetes was controlled for 10 consecutive days. Upon resuming commercial insulin (3 doses) the blood sugar rose and glycosuria reappeared.

Comment. This patient received 24 injections of insulin tannate totalling 19 mg. of tannic acid. Erythema about the site of injection was not encountered. Although subcutaneous lumps occasionally resulted, these were not troublesome to the patient. Insulin tannate sufficed to control this case of diabetes when used alone. Increased efficiency of insulin tannate over commercial insulin is shown by the facts that 2 doses replaced 3 of commercial insulin and that fewer total units were necessary.

Case 2. H. V., a boy aged 13 years, had had diabetes for 2 years. Examination revealed a poor posture, malnutrition, and a well compensated mitral stenosis. Throughout the period of observation to be described, his diet consisted of C-240, P-82, F-121, Cal. 2377. He always ate all of his food. The total 'glucose value' of his diet varied only 7 gm. throughout the experiment. His body weight varied from 51.1 to 51.6 kg. He exercised each day at the same hour and to about the same degree.

Before the experiment the patient had been sugar-free 9 consecutive days. His dose of commercial insulin was then lowered sufficiently to permit a mild glycosuria. After a few days on 10 u. of commercial insulin twice daily he passed 13.7 to 16.1 gm. of urine sugar, and the fasting blood sugar was 168 to 170 mg. per cent. Insulin tannate alone was then given (10 u. twice daily) for 4 consecutive days. Immediately both the blood and urine sugar decreased. On the fourth day the fasting blood sugar was 158 mg. per cent and the urine was sugar-free. Upon returning to commercial insulin, sugar reappeared in the urine. The effect on the blood sugar lasted several days.

A second experiment was performed after an interruption of a few days. For 4 days on 11 u. of commercial insulin twice daily the blood sugar fasting was 164 to 174 mg. per cent, and the urine sugar varied from 8 to 13.6 grams. When insulin tannate alone was used this time no effect was observed. The blood sugar did not fall and the urine sugar increased. During this 3-day period some skin reactions occurred. These were large, painful subcutaneous lumps at the site of injection of the insulin tannate. The overlying skin was red. Culture of the bottle of tannic acid revealed a growth of mould-like organisms. Upon substituting a fresh bottle of proven sterility the fasting blood sugar promptly fell and after 4 days the urine contained but a trace of sugar. The appearance of a hypoglycemic reaction necessitated lowering the total unitage of insulin tannate. As the graph shows, an attempt was made to reduce the unitage to a minimum. On the dosage plan of 2 u. of insulin tannate in the morning and 11 u. in the evening, a more satisfactory state of diabetic control could be maintained than on 2 doses of 11 u. each of commercial insulin. One dose of 11 u. of insulin tannate given 2 hours before supper (the major meal at the Santa Barbara Cottage Hospital) sufficed to maintain the fasting blood sugar normal the next day but did not control glycosuria. Upon returning to commercial insulin of the same unitage as used toward the end of the experiment, the fasting blood sugar and urine sugar promptly rose.

Comment. Chart 2 shows clearly that a diabetic child was adequately controlled for 3 consecutive weeks on insulin tannate. When sterile solutions were used no skin reactions were noted. Subcutaneous lumps occasionally persisted at the site of injection of the solution for 24 hours. These were rarely painful. In all, this patient received 51 injections of insulin tannate representing a total of 16.6 mg. of tannic acid. He was conscious of a greater 'sting' during the injection but never considered

such lumps as occurred as any inconvenience. The ratio of insulin to tannic acid in the solutions used varied from half-saturation to an excess of free tannic acid. During the latter part of the experiment when the unitage was reduced, it was done by reducing the volume (hence the units) of insulin and leaving constant the volume of tannic acid solution mixed with it. The patient received then on 2 occasions, 0.02 cc. of U-100 insulin plus 0.11 cc. of tannic acid solution (3 mg. per cc.). In spite of an excess of free tannic acid present in such a mixture no skin reactions followed its injection.

Case 3. C. K., a well developed male, aged 49 years, had had diabetes for 6 years. He had been observed at various intervals over a period of 5 years and had never been consistently sugar-free. Before beginning the experiment he required up

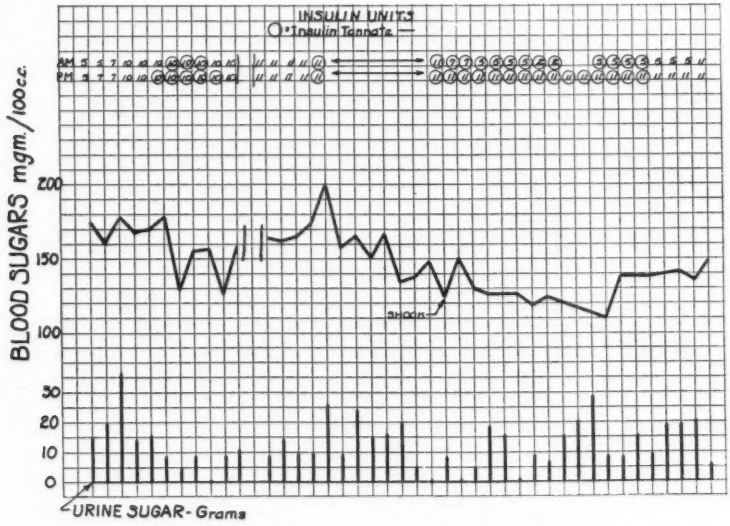

Chart 2. Case 2 (H. V.). Note the lower fasting blood sugar values when insulin tannate was used. Augmented effect is also shown by a lesser total amount of insulin tannate being adequate to control the diabetes.

to 100 u. of commercial insulin daily in order to keep from passing more than 10 gm. of urine sugar. His diet remained constant throughout the experiment. All of his food was eaten each day. His body weight varied from 57.2 to 57.7 kg. He was allowed to be about the ward with occasional walks in addition.

For 5 days his insulin dose varied from 89 to 100 u., divided into 4 doses. Glycosuria varied from 5 to 21 gm. daily. The fasting blood sugar varied from 300 to 338 mg. per cent. The 5:00 P.M. blood sugar varied from 130 to 196 mg. per cent. On February 13, 1936 an attempt was made to control his diabetes with insulin tannate alone. Fearful lest insulin shock should appear, we reduced the total units for the day to 75. On the following day (2-14-36) shock did appear at 9:00 P.M. Insulin tannate alone was used in this case for 14 consecutive days. During the first week the glucosuria and blood sugar values were of about the same magnitude as with commercial insulin. During the second week the glycosuria was about the same but the blood sugar values were slightly higher. The fasting blood sugar varied from 250 to 338 mg. per cent, and the 5:00 P.M. blood sugar from 112 to 322 mg. per cent. Glycosuria varied from 8 to 40 gm. daily. However, the variation

between the fasting and the 5:00 P.M. specimens was less than on commercial insulin. Upon resuming commercial insulin glycosuria increased and the wide fluctuations in the blood sugar reappeared.

Comment. Two doses of insulin tannate controlled this case almost as well as 3 to 4 doses of commercial insulin. Various dosage schemes were tried. The best one in this particular case seemed to be about 55 per cent of the total units 1 hour before breakfast and the remainder one-half hour before supper. The fasting blood sugar the following morning did not seem to be lower when insulin tannate was given after instead of before supper. During the first week of the experiment the patient received a total of 20 per cent less units of insulin tannate than of commercial insulin. This gain disappeared in the second week. Some of this apparent loss may have been due to constant changing of the time of the injections.

This patient received 28 injections of insulin tannate, representing a total of 35 mg. of tannic acid. No local erythema or subcutaneous lumps appeared. He was not inconvenienced by the injections.

Case 4. H. T., a boy aged 14 years, had had diabetes for 4 years. He had never been consistently sugar-free in spite of large doses of insulin, and he had been in coma several times. Throughout the experiment his diet remained constant (C-299, P-88, F-73, Cal. 2205). His exercise was about the same each day. He was found to be particularly susceptible to insulin shock in the late afternoon in spite of high fasting blood sugar levels. On 2 successive occasions for 6 and 9 consecutive days respectively, insulin tannate was used to control his diabetes. Two doses of the new insulin controlled his diabetes as well as 3 to 4 doses of the old. Occasional subcutaneous lumps appeared, but these were ephemeral.

This patient received a total of 26 injections of insulin tannate containing 14.3 mg. of tannic acid. No local reactions were encountered.

Case 5. E. W., a female child aged 6 years, had had diabetes for 4 years. She had been in coma on several occasions. She was admitted to the hospital in ketosis complicated by interlobar pleurisy. One month after admission the child had completely recovered. She was free of fever and played about the ward. Throughout the experiment to be described her diet was constant and she always ate all of her food. The formula was C-161, P-74, F-63, Cal. 1417. Although she was not confined to bed, her exercise from playing did not vary materially from one day to another. Her body weight varied from 20.6 to 21.1 kg. during the experiment.

After she had been sugar-free for a week her insulin dosage was lowered sufficiently to allow some glycosuria. Capillary blood sugar specimens were taken daily at 6:00 A.M. and 4:30 to 5:00 P.M. At first 8 u. of commercial insulin were given before breakfast and 4 u. before lunch and 16 u. of insulin tannate before supper. On this program glycosuria disappeared. Each night for the first 3 nights hypoglycemic reactions were encountered. On the third night one occurred sufficiently severe to require an intravenous injection of glucose. The insulin tannate was then lowered to 12 u. During the next 5 days the fasting blood sugar (capillary) values varied from 360 down to 80 mg. per cent and the afternoon levels from 360 to 240 mg. per cent. For 2 days after insulin tannate was discontinued the fasting blood sugar of the following day remained low; subsequently, however, it rose.

In the second phase of the experiment insulin tannate was used alone. Hyperglycemia and glycosuria were not controlled. However, only 2 doses were given each day. These did about as well as 3 doses of commercial insulin, but not quite so well as 4 such doses. In all, this patient received 21 doses of insulin tannate, representing 7.8 mg. of tannic acid. Only an ephemeral erythematous flush was ever encountered

at the site of injection of insulin tannate, and then only occasionally.

In this case a combination of commercial insulin and insulin tannate proved to be more effective than either alone. The commercial insulin was given before breakfast and lunch, and insulin tannate was given before supper. Such a program obviated the necessity of giving a dose of insulin during the night.

Case 6. M. M., a girl aged 15 years, had had diabetes of maximal severity for 8 years. She had been in coma on several occasions. Her case had been followed by us continuously since the onset of the diabetes. Glycosuria and hyperglycemia had never been controlled in spite of large doses of insulin. Prior to the experiment to be described, her insulin requirement had been between 95 and 115 u. daily, given

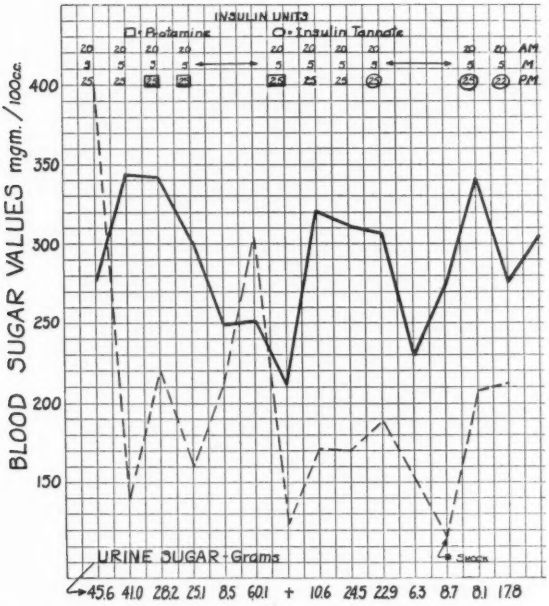

Chart 3. Case 7 (F. M.). Protamine insulinate and insulin tannate, when given before supper in conjunction with commercial insulin before breakfast and lunch, produced lower blood and urine sugar values. The solid line represents the fasting and the dotted line the 5:00 P.M. blood sugar values.

in 4 doses. During the period of observation her diet and exercise were constant. The diet formula was C-160, P-73, F-82, Cal. 1670. Two doses of insulin tannate sufficed to control her diabetes better than did commercial insulin. The average fasting blood sugar for 3 consecutive days before the experiment when commercial insulin had been given the preceding evening, was 335 mg. per cent. For the 9 consecutive days when insulin tannate had been given the preceding evening, it was 242 mg. per cent. Immediately upon discontinuing insulin tannate and substituting for it the same number of doses and the same unitage of commercial insulin, glycosuria and hyperglycemia increased.

To date, this patient has received 21 injections of insulin tannate, totalling 22.2 mg. Painful subcutaneous lumps appeared when the salt was injected into the legs, but not the arms.

INSULIN TANNATE VERSUS PROTAMINE INSULINATE

In 2 of the patients already cited and in 3 additional ones, a comparison has been made between the effect of insulin tannate and that of protamine insulinate. The method first used was that suggested by Hagedorn (3) and Root (4). Commercial insulin was given before breakfast and before lunch, when a noon dose was necessary, and the new insulin preparation before dinner. Three cases were tested to both preparations according to this scheme. They received a constant diet and constant unitage of insulin throughout the period of observation. All 3 cases showed lower levels of blood sugar fasting on the mornings after evening doses of either insulin tannate or protamine insulinate. The fall in the fasting blood sugar was of about the same magnitude with both preparations. In the other 2 patients in the group insulin tannate and protamine insulinate were used alone to control the diabetes at different periods, with control periods of commercial insulin between them. Again the fasting blood sugars, during the periods when the new preparations were used, averaged lower than during the control periods and glycosuria was less. The change was also of about the same magnitude for each preparation.

Case 6. M. M. (continued). The patient, M. M., cited above, received in a second experiment 2 doses of commercial insulin during the day and 1 dose of the new insulin at bedtime. The total unitage for the day was so arranged as to remain constant (with only 1 exception). When she received 85 u. of commercial insulin (50 u. before breakfast and 35 u. before supper) the fasting blood sugar averaged 389 mg. per cent. When she received 2 doses of commercial insulin (45 u. before breakfast and 35 u. before supper), and 5 u. of protamine insulinate at bedtime, the fasting blood sugar averaged 370 mg. per cent. When 5 u. of insulin tannate were substituted for the protamine insulinate the fasting blood sugar averaged 375 mg. per cent. Glycosuria was less during the insulin tannate period than during the protamine insulinate period.

Case 7. F. M., a male aged 58 years, had had diabetes 16 years. Throughout the experiment he remained at bed rest and consumed all of his food. Blood sugar specimens were taken twice daily. Before the new insulin was administered his dose of commercial insulin was adjusted to permit a glycosuria of 20 to 40 gm. The total unitage for the day was kept constant. Commercial insulin was given before breakfast and lunch, and the new insulin before supper. During the control periods his fasting blood sugar level averaged 317 mg. per cent. While he was receiving protamine insulinate it average 267 mg. per cent and while receiving insulin tannate, 286 mg. per cent. Glycosuria in both experimental periods exceeded that of the control periods (chart 3).

Hypoglycemic shock sufficient to require 50 per cent glucose intravenously occurred at the point designated on the chart. The following day the units of insulin tannate were reduced. In order to test this patient's tolerance on this reduced dosage he was put back on 3 doses of commercial insulin in the new unitage, after a few days' interval during which other experimental observations irrelevant to this report were being made. The fasting blood sugar level promptly rose.

Case 8. S. H., a male aged 73 years, had had diabetes for 18 years. He was admitted to the Santa Barbara Cottage Hospital with a necrotic ulcer on the foot, which was treated conservatively and eventually healed. During the course of the experiment he was at continuous bed rest and free of temperature. His carbohydrate

tolerance had been stationary for 3 weeks before beginning the experiment. His diet was constant, and he ate all of his food.

On February 17, 1936 2 doses of insulin tannate were substituted for 3 doses of commercial insulin. On the second day a hypoglycemic shock appeared and the unitage had to be lowered. After 7 days this case was controlled on 2 doses of insulin tannate, representing 20 per cent less units than formerly on 3 doses of commercial insulin. In all, 14 doses of insulin tannate containing 9.6 mg. tannic acid were given. No cutaneous lesions were encountered.

An attempt was then made to compare the effect of insulin tannate to that of protamine insulinate. On February 26, 1936, 31 u. of commercial insulin were given before breakfast and 12 u. of protamine insulinate before supper. A fall in the fasting blood sugar was noted at once. On the 3rd and 4th of March 1 dose of

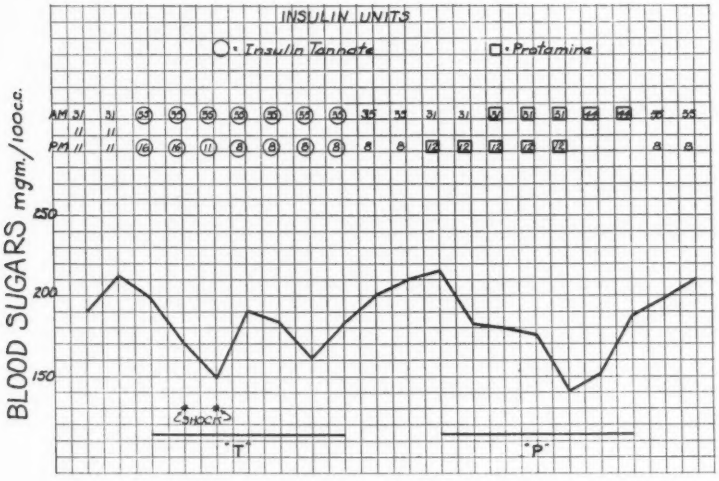

Chart 4. Case 8 (S. H.).

protamine insulinate (44 u.), given 2 hours before breakfast, sufficed to maintain the blood sugar at its usual level the ensuing morning. Upon returning to commercial insulin in equal unitage the blood sugar rose and glycosuria reappeared (chart 4).

The average blood sugar fasting (7 consecutive days) while this patient was taking 43 u. of insulin tannate was 176 mg. per cent. With 44 u. of protamine insulinate it was 170.5 mg. per cent. For 2 consecutive days 1 dose of protamine insulinate sufficed for 24 hours. One dose of insulin tannate was not tried.

Case 9. J. B., a well developed male aged 32 years, had had diabetes for 5 years. He had never been consistently sugar-free. Throughout the experiment his diet remained C-295, P-86, F-74, Cal. 2190, and his insulin dose remained 50 u. in the morning, 10 u. at noon, and 20 u. in the afternoon. On this program during the control periods when commercial insulin was used, his fasting blood sugars varied from 338 to 372 mg. per cent (average, 353), and he passed from 18 to 49 gm. of sugar daily. On one occasion 20 u. of insulin tannate were substituted for commercial insulin for the evening dose for 4 consecutive days. During this period his fasting blood sugar level varied from 302 to 330 mg. per cent (average, 322) and he passed from 16 to 30 gm. of urine sugar. Upon another occasion, 20 u. of prota-

mine insulinate were substituted for the evening dose for 4 consecutive days. During this period, his fasting blood sugar levels varied from 314 to 388 mg. per cent (average, 343), and he passed from 39.6 to 66.4 gm. of urine sugar.

Comment. Insulin tannate in this case yielded lower levels of fasting blood sugar and less glycosuria than did either the commercial insulin or the protamine insulinate. No hypoglycemic reactions or skin reactions were encountered with either preparation. Both were given at the same time of day, but the insulin tannate was given three-quarters of an hour and the protamine insulinate 2 hours before supper. This difference in timing may explain the difference in glycosuria noted.

DISCUSSION

By applying to insulin the important principle of precipitating protein hormones, discovered by Bischoff and Maxwell, a new preparation has been developed which possesses certain advantages over the commercial insulin now in use in the treatment of diabetes mellitus. These advantages depend upon its delayed but prolonged hypoglycemic action. Fewer doses per day and less total unitage suffice to control the diabetes. The incidence of hypoglycemic shock seems to be less, although this is merely a clinical impression. When insulin tannate is given before supper the fasting blood sugar of diabetic children is lower the following morning. It has obviated the necessity of a midnight dose of insulin in children in order to control their metabolism during the night. Before working with insulin tannate we had despaired of ever keeping the fasting blood sugar level of our severely diabetic children within reasonable limits without a dose of insulin given during the night or early morning.

In its present state of development Bischoff and Maxwell's preparation calls for 2 vials of solution, one containing U-100 insulin and the other a tannic acid solution. Since only one brand and strength of commercial insulin has been used, statements regarding the chemical characteristics of the mixture apply only when that particular brand has been used. Competing brands of commercial insulin contain different diluents. Conceivably, differences in the salt resulting from mixing them with tannic acid would result. This question merits further study.

A routine time schedule for the injection which would be applicable to all patients has not yet been satisfactorily worked out. The time of the doses in relation to the subsequent meals and the proportion of the total day's dose to be given at each injection seems to be an individual problem. When 2 doses of insulin tannate were used the larger dose, as a rule, was given from one-half to 2 hours before breakfast and the smaller dose the same interval before supper. The cases which we have studied to date have been mostly severe ones, requiring 3 to 4 doses of commercial insulin daily. Such cases were selected because we thought that any improvement over their regular insulin program would be apparent quickly and also because it is in this type of diabetic patient that the need for improvement in therapy is most urgent. Tannic acid is cheap and readily available. Its use in combination with commercial insulin will not increase the cost of the medicine to the patient. This is an obvious advantage over protamine insulinate. At the present time the

continued availability of protamine in amounts sufficient to supply the demand and at a cost not prohibitive to the patient, remains to be demonstrated. Our limited experience with these 2 preparations leads us to believe that they both possess the same advantages and the same disadvantages, except for this question of cost and supply. Both prolong the action of insulin, reducing the number of injections required per day and reducing the number of units required. Both can be used to treat diabetes, alone or in combination with other doses of commercial insulin. Both produce subcutaneous lumps and skin reactions in susceptible individuals. From personal, unpublished observations on the factors in commercial insulin which produce skin reactions, we believe that this problem of local reactions can be solved by further chemical research. The prolongation of the action of either insulin tannate or protamine insulinate is apparently not dependent upon the production of subcutaneous lumps, because the effect is present in those patients who do not exhibit such lumps. From the mass of precipitated insulin, free insulin is probably liberated in small amounts over a period of from 12 to 15 hours. A word of caution is in order here. This delayed liberation of insulin may coincide with a low blood sugar produced by a subsequent injection of commercial insulin, producing profound shock.

INSULIN-ZINC

Since certain heavy metals are also effective protein precipitants, the hypoglycemic effect of their salts with insulin should be similar to that of the organic precipitants, protamine and tannic acid. To date 4 patients have received insulin-zinc compound, containing 0.1 mg. zinc per 100 u. of insulin. No local reactions have been encountered. When used in conjunction with regular insulin, improvement in diabetic tolerance has been noted. Fasting blood sugar levels have been found to be lower when insulin-zinc is given at night than when the same number of units of regular insulin are given, and in all 4 patients fewer total units per day have sufficed to control the metabolism. Insulin-zinc has been shown to maintain normal blood sugar levels for at least 10 hours after a single injection in diabetic patients. The value of insulin-zinc in controlling selected cases of diabetes when used alone and a comparison of its hypoglycemic effect to that of insulin tannate and protamine insulinate will comprise a subsequent communication.

SUMMARY

Insulin tannate is salt of insulin which is insoluble in the presence of sodium chloride at a pH acid to the iso-electric point. It is prepared by mixing in a hypodermic syringe equal volumes of U-100 strength insulin and a tannic acid solution. The resulting mixture contains 3 mg. of tannic acid per 100 cc. It has about 20 to 25 per cent more hypoglycemic action per unit than commercial insulin.

Eighteen patients of various ages and with diabetes of varying degrees of severity have been treated with insulin tannate for periods varying from a few days to 2 weeks consecutively. Control of the diabetes has been possible when this compound is used alone or in combination with commercial insulin. Be-

cause of the relative insolubility of insulin tannate in human and animal tissues, delayed absorption and hence prolonged liberation of free insulin occurs. Increased effectiveness of insulin tannate over commercial insulin is shown by lower fasting blood sugar levels and smaller number of total units required per day when it is used. The hypoglycemic effect of insulin tannate has been compared to that of protamine insulinate and found to be of about the same order. The cheapness and availability of insulin tannate recommend it.

I wish to thank Dr. Newell Nay for his assistance in the preparation of the exhibits.

REFERENCES

1. Bischoff, F., and L. C. Maxwell: Am. J. Physiol. **112:** 172. 1935.
2. Bischoff, F., and L. C. Maxwell: Am. J. Physiol. (In press).
3. Hagedorn, H. C., B. N. Jensen, et al.: J.A.M.A. **106:** 177. 1936.
4. Root, H. F., P. White, et al.: J.A.M.A. **106:** 181. 1936.

THE EFFECT OF OESTROGENIC HORMONE ON EXPERIMENTAL PANCREATIC DIABETES IN THE MONKEY[1]

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While the participation of some extra-insular factor in pancreatic diabetes had been suspected for many years it remained for Houssay and Biasotti (1), as a climax to a long series of experiments, to demonstrate that the anterior hypophysis plays an important role in carbohydrate metabolism. They were able to show that pancreatectomy in the hypophysectomized toad or dog as a rule is not followed by the usual sequelae of pancreatic deprivation. These animals instead of dying within a few days of acute diabetes often survive for many months. Confirmation of Houssay's studies has appeared from several sources (2, 3, 4, 5) so that it would appear to be well established that the anterior pituitary secretes a hormone which in some fashion elevates the blood sugar. The modified form of diabetes observed in hypophysectomized animals suggested the possibility of securing the same result by suppressing the 'diabetogenic'[2] factor with oestrone. This procedure, based on the demonstration (6, 7, 8) that oestrone exerts a suppressing influence on the gonadotropic and lactogenic activities of the hypophysis, was first carried out on the dog (9). During the period after pancreatectomy when oestrone (Amniotin in oil, 200 R.U. daily) was injected the glycosuria was mild. Withdrawal of oestrone precipitated a severe glycosuria in each instance; when treatment was resumed the glycosuria was again controlled. The present study deals with an extension of this procedure to the rhesus monkey.

EXPERIMENTAL PROCEDURE

Eleven sub-adult females and 1 young male, weighing from 2.7 to 3.8 kg., were used. They were kept in specially constructed metabolism cages during the period of study and received the usual colony ration of bananas, oranges, and dog chow. Urine was collected as 24-hour samples. All blood samples were taken in the morning before the animals were fed. The method of Shaffer and Somogyi (10) was employed for both blood and urine sugar analyses. We have studied the urine content of non-fermentable reducing substances in

[1] This investigation was aided by a grant from the Committee on Scientific Research of the American Medical Association to Dr. M. D. Overholser.

[2] The term 'diabetogenic' to designate the activity of the anterior hypophysis in relation to carbohydrate metabolism leaves much to be desired. This phase of pituitary function is active in the normal individual without giving rise to a diabetic condition. It would appear that 2 pituitary principles may be concerned in carbohydrate metabolism (16). One of these is believed to increase the blood sugar level, the other to have ketogenic activity.

representative samples and have observed regularly a daily output of about 100 mg. Pancreatectomy was performed under aseptic conditions, with ether anesthesia, and by a transperitoneal approach.

As an initial procedure we injected several pituitary extracts into 2 normal monkeys. Two of these preparations, one an alkaline extract of sheep pituitaries and the other an acid extract of beef glands, were shown to possess some blood sugar raising activity when injected intraperitoneally in amounts of 15 cc. daily for 4 days. Fasting blood sugars were elevated 35 to 60 mg. per cent and 0.4 to 0.8 gm. of sugar per kilogram of body weight was excreted daily. Polyuria was present to a mild degree.

Ten animals were depancreatized. Two of the females received 100 R.U. of oestrone[3] daily for 15 days, and the one male received 300 R.U. weekly for about 7 months, prior to pancreatectomy. The remaining animals were not treated before the operation. In each instance at least one pre-operation blood sugar was determined. While individual animals have shown fairly consistent values we have observed considerable variability between different animals (55 to 125 mg. per cent).

The general procedure adopted for all depancreatized animals, with the exception of those receiving oestrone prior to operation, has been to observe the urine and blood sugars for several days following pancreatectomy. At this time blood sugars of 300 to 400 mg. per cent and urine sugars of 2 to 3 gm. per kg. of body weight established the diabetic condition of the animals. Injections of oestrone were then begun and continued for varying periods of time. The dosage ranged from 100 to 500 R.U. daily in different instances, but there is little evidence that 500 R.U. has been more effective than 200 R.U. in these experiments.

RESULTS

In monkeys 1 and 2 sub-total pancreatectomy was performed. These animals showed only mild hyperglycemia (170 to 200 mg. per cent) and glycosuria (1.2 to 1.8 gm. per kg.) during the post-operative period. The glycosuria was almost completely abolished and the blood sugar level lowered to about 130 mg. per cent within 6 days after injections of oestrone were begun. Cessation of treatment was followed by a rise in the blood and urine sugars and its resumption by a corresponding decline. These animals were kept under continuous observation for 70 days. During the latter part of this period it became apparent that they were recovering from the relatively mild diabetes they had experienced. Approximately a year later they were used in an experiment of a different nature.

In the remaining 8 cases pancreatectomy was complete. This was indicated by the blood and urine sugar findings and was assured at autopsy. The survival periods of the 7 animals that were injected with oestrone varied from

[3] The oestrone used in these experiments was either theelin in oil, supplied by Dr. Oliver Kamm of Parke, Davis & Co., or Amniotin in oil, furnished by Dr. J. A. Morrell of E. R. Squibb's Sons. We have been unable to detect any difference in the activity of equivalent amounts of these preparations and consequently do not refer to the preparations used in individual experiments. It should be noted that we have uniformly conducted our own assays on each lot of material received for use. Our rat unit is that minimum amount of oestrone which will induce full vaginal cornification in at least 7 of 10 spayed rats. The oestriol (theelol) was supplied by Dr. Kamm and contained 50 R.U. per kapseal. The writers wish to express their gratitude for the large amounts of these preparations that were placed at their disposal.

18 to 216 days. The one uninjected control, monkey 7, died on the eighth day after pancreatectomy. She excreted increasing amounts of sugar up to the seventh day when 5.1 gm. per kg. were present in the urine. Of the 7 animals that received treatment with oestrone those showing the longest survival periods were monkeys 5 and 17 which had been injected daily for 15 days prior to and for 6 days following pancreatectomy. In these 2 animals only a mild glycosuria (the maximum was 0.5 gm. per kg.) occurred during the week immediately following operation. When treatment was discontinued the urine sugar increased to about 3.5 gm. per kg. The resumption of injections was followed by a gradual decline in the glycosuria and alternate periods of injections and withdrawal of injections resulted in corresponding fluctuations in the urine sugar level.

Monkey 17 lived for 72 days following pancreatectomy, succumbing to metastatic abscesses in the liver and lungs. Her diabetes had been well controlled and her general condition had remained excellent up to a few days before death. Monkey 5 continued in excellent condition for about 190 days after pancreatectomy. Prior to this time she had not excreted more than 1.1 gm. of sugar per kg. per day during her periods of treatment with oestrone. However, a gradual increase in glycosuria became apparent and despite an increased amount of oestrone she excreted 2.5 to 3.0 gm. of sugar per kg. and showed a blood sugar of 370 mg. per cent. Cessation of treatment was followed by a rise in the blood sugar to 585 mg. per cent and an elevation of the urine sugar to 5.5 gm. per kg. When treatment was resumed the urine and blood sugars were again transiently, but materially, reduced for a few days. Despite the administration of increasing amounts of oestrone her condition became progressively worse. Beginning on the 212 day after operation and continuing for 3 days she was injected with 10μ of insulin twice daily, the first she had received. This served to reduce her blood sugar materially and greatly decreased the urine sugar but her condition had become so perilous that she was sacrificed on the 216th day after pancreatectomy. Autopsy showed pancreatectomy to have been complete. Figure 1 shows the fluctuations in the urine sugar over the entire post-operative life of monkey 5.

The five remaining animals showed less striking responses to treatment. Monkey 11, a young adult male, had received 300 R.U. of oestrone a week for 7 months prior to pancreatectomy. Despite the continuation of treatment (200 R.U. daily) after the operation he excreted 1.6 to 2.4 gm. sugar per kg. per day. Cessation of treatment for 3 days was followed by a rise to 4.2 gm. per kg. The resumption of injections (500 R.U. daily) decreased the glycosuria to about 2.0 gm. per kg. Although his general condition remained fairly good throughout he died suddenly on the 34th day after pancreatectomy.

Monkey 8 showed very little improvement with oestrone treatment (200 to 300 R.U. daily). On the 10th day after operation her urine sugar attained the high figure of 6.8 gm. per kg. and the blood sugar was 575 mg. per cent. The injection of 20 μ of insulin reduced the urine sugar to 1.4 gm. per kg. on the 12th day, and after a subsequent rise on the following 3 days when no insulin was given, to 1.6 gm. on the 16th day. The animal died in typical diabetic coma on the 19th day.

Monkey 9 responded well to treatment and on the 10th day after operation her urine showed only 0.5 gm. sugar per kg. On the 11th and 12th days she refused to eat. Throughout the next week, during which she refused food, she was given 20 to 30 gm. of sugar in milk by stomach tube each day. Despite the considerable amounts of sugar so administered the largest amount recovered in a single 24-hour

sample of her urine was 1.3 gm. per kg. On the 18th day she was found in convulsions and despite several intravenous administrations of glucose she died on the following day.

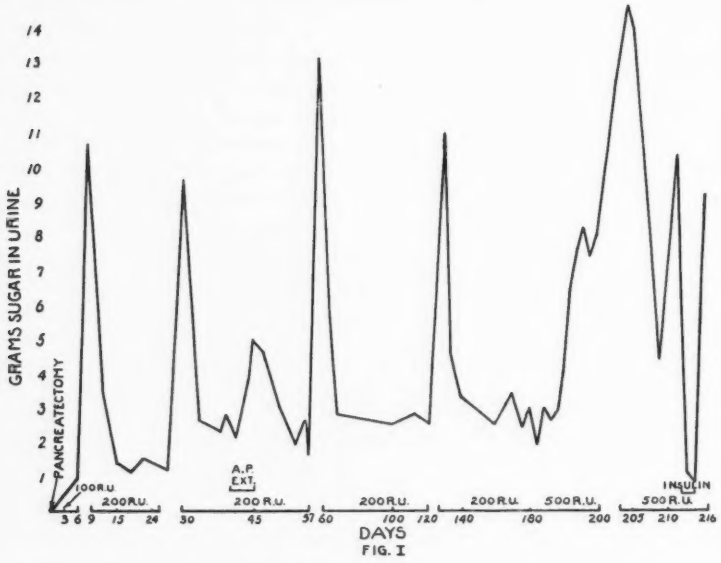

FIG. I

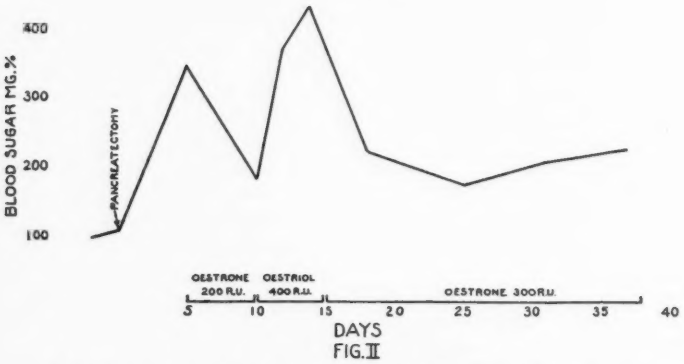

FIG. II

Fig. 1. Urine sugar readings for monkey 5. Fig. 2. Blood sugar readings for monkey 10.

Monkey 10 showed a urine sugar of 2.8 gm. per kg. on the 5th day after pancreatectomy. At this time injections of oestrone (200 R.U. daily) were begun. The glycosuria increased to 4.1 gm. per kg. on the 6th day and then decreased progressively until the 10th day when only 0.3 gm. per kg. was present in the urine. Oestrone was then discontinued and the administration of oestriol (theelol) by mouth was begun. This treatment, 8 kapseals daily, was continued for 5 days. Throughout this period the urine sugar increased progressively to 4.8 gm. per kg. Oestriol was then discontinued and injections of oestrone (300 R.U. daily) were

resumed. The urine sugar decreased to 0.8 gm. per kg. on the 18th day and remained, with minor fluctuations, at that level until the 33rd day. A slight increase (1.1 to 1.4 gm. per kg.) occurred during the following 5 days. Although this was not looked upon as a serious development she was found dead on the morning of the 39th day.

Monkey 12 showed a glycosuria of 3.8 gm. per kg. on the 4th day after operation when injections of oestrone were begun. Despite an increase in the dosage of oestrone (from 200 to 400 R.U. daily) the glycosuria remained high, fluctuating between 3.4 and 4.8 gm. per kg., until the 18th day when the animal's condition led us to sacrifice her.

During one of the periods while monkeys 5 and 17 were under treatment with oestrone several pituitary extracts were injected. As previously noted (11) one of these preparations caused an increased glycosuria.

It was not possible to make as frequent tests for the blood sugar levels as we would have desired because of the considerable difficulty encountered in obtaining blood from small monkeys. The average level prior to operation was about 85 mg. per cent, the diabetic level about 450 mg. per cent, and the level during successful oestrone treatment about 190 mg. per cent. On monkey 10 a fairly complete series of blood sugar readings during her life after pancreatectomy was secured. These readings are reproduced in figure 2.

The body weights of the animals were followed closely and it was noted that during periods of severe glycosuria considerable weight was lost. On the other hand, weight increases occurred during periods of successful treatment. The usual reddening of the sexual skin was observed during the relatively long periods of treatment with oestrone and bleeding was noted in such instances when the injections were discontinued. At autopsy liver, gut, stomach, adrenal, thyroid, hypophysis, testis, ovary, uterus, seminal vesicle, prostate and mammary gland were regularly collected for study. If any fragments of issue were found in the pancreatic region they too were preserved. Examinations for pancreatic tissue have been uniformly negative. The study of the above tissues has revealed little of significance except in the reproductive organs and the hypophysis. The ovaries, particularly in the animals that survived longest, were reduced in size and contained only small follicles. The testes of the one male were less than half the size normal for animals of his age range and the seminal vesicles were very small. The pituitaries of all injected animals showed a type of granular loss in the basophiles similar to that which has been observed in the hypophysis of the rat after injections of oestrone (12). The mammary glands of monkeys 5, 11, and 17 showed a considerable degree of development. Not only was there an extension of the duct system, but also an appearance of numerous alveoli arranged in definite lobular patterns. This observation is of considerable interest in its bearing on the relation between oestrone and progesterone in the control of mammary development (13).

DISCUSSION

Although it would have been of interest, a thorough consideration of the nitrogen and ketone body elimination in our animals was not feasible. However, the striking modification of the blood and urine sugar levels and the

prolongation of the lives of animals treated with oestrone has been impressive. Usually, although not invariably, the sugar levels of the blood and urine decreased within 2 days after the initiation of treatment with oestrone. Correspondingly, increased sugar levels occurred on the third or fourth days following the cessation of injections. It should be emphasized that the oestrone used in these studies was an oil preparation. We have considerable reason to believe, from other experiments, that oestrone in oil is far more effective than equivalent or even much larger doses of aqueous oestrone. The one experiment, on monkey 10, conducted with oestriol by mouth showed no effectiveness on the part of that preparation.

We have interpreted the modified diabetes observed in animals treated with oestrone on the basis of a suppression of the 'diabetogenic' activity of the hypophysis. This seems to be the most reasonable explanation in view of the demonstrations that hypophysectomy has a modifying influence on experimental diabetes and that oestrone exerts a suppressing influence on the gonadotropic and lactogenic activities of the hypophysis.[4] In the present experiments the gonads showed evidence of a lack of stimulation by gonadotropic hormone. Consequently it would seem logical to suppose that the modification of the diabetic condition of the animals was due to something in the nature of a functional hypophysectomy. Furthermore, the injection of a pituitary extract in animals whose diabetes was controlled by oestrone resulted in increased sugar levels in the blood and urine.

Although only a few animals survived more than 30 days it is significant that each of the injected animals lived at least 10 days longer than the one control. The average survival period of the 7 totally depancreatized monkeys that had received oestrone treatment was 69 days. If monkey 5, which lived 216 days, is eliminated the average duration of life after pancreatectomy was 33 days. This would appear to be a significant effect and is somewhat comparable to the usual survival period of Houssay animals or of the adrenalectomized-depancreatized preparations of Long and Lukens (14). Survivals of as much as 6 months, even on the part of Houssay animals, are exceptional.

The manner in which the hypophysis exerts its effect on carbohydrate metabolism is still somewhat obscure. Houssay and his co-workers believe that the hypophyseal effect is a direct one. Barnes, Scott, Ferrill, and Rogoff (15) consider the adrenal medulla to be an intermediary factor, and Long (16) has advanced evidence in favor of the action of the pituitary through the adrenal cortex. Although the evidence presented by Long and his associates seems particularly impressive, we have noted no indication of any effect of oestrone on the histology of the adrenal cortex in our experiments. However, we would be unwilling to conclude that the adrenals have not been involved in the effects we have observed.

Long has pointed out that the removal of the 'contra-insular' factors, i.e.,

[4] Some qualification of this statement concerning the inhibition of the gonadotropic activity of the hypophysis is necessitated by certain recent reports. Hohlweg (14), Lane (15), Selye, Collip and Thomson (16), and Nelson (17) have presented evidence obtained on female rats injected with oestrone which finds its most logical interpretation on the basis of a release of luteinizing hormone from the pituitaries of the injected animals. However, all evidence thus far presented has pointed to an inhibition of the follicle stimulating hormone by oestrone. Indeed, the stimulating action of oestrone on the release of luteinizing hormone has been demonstrated in the rat alone.

the anterior pituitary, the adrenal cortex, or both, serves only to modify the destructive conversion of the tissue proteins into glucose and the attendant accumulation of the products of fat metabolism. Thus, hypophysectomy or adrenalectomy in depancreatized animals does not reinstate normal carbohydrate metabolism. The latter process is not possible in the absence of insulin. On the other hand, Houssay and Biasotti (1), while admitting a reduction in the conversion of endogenous proteins into glucose by Houssay animals, consider that an additional factor in the improved condition of these animals may be an actual increase in the utilization of sugar. In this regard it is of interest to note that while we have not conducted sugar tolerance tests in our present studies, we have in 2 instances given 10 gm. of sugar by stomach tube to monkeys whose diabetes was under control with oestrone. Only a fraction of this sugar was recovered in the urine. Furthermore, one animal (monkey 9) that had received 20 to 30 gm. of sugar daily as her only food for 8 days excreted only a small amount of the ingested sugar in her urine.

Although the clinical use of oestrone in diabetes has not been reported we have received a number of communications concerning its trial. Some of these reported encouraging results, while others were entirely negative. It seems probable that the greatest chance for clinical success would be in the use of oestrone in individuals showing disturbances of carbohydrate metabolism which might be attributed to a pituitary disorder, e.g., acromegaly or pituitary 'basophilism'. However, in view of the strong case Long has made for the existence of a diabetic condition in the Houssay animal, despite the relatively mild character of the disease, hypophysectomy or the inhibition of the hypophysis with oestrone cannot be looked up as a substitute for insulin in the regulation of deficiencies of carbohydrate metabolism.

SUMMARY

The possibility of controlling experimental pancreatic diabetes by the injection of oestrone in oil has been studied in the rhesus monkey.

Two normal monkeys showed hyperglycemia and glycosuria after the administration of crude pituitary extracts for several days. In two monkeys a relatively mild diabetes occurred after sub-total pancreatectomy. The administration of oestrone reduced the hyperglycemia and almost abolished the glycosuria in these animals.

Eight monkeys were totally depancreatized. The one untreated control animal died on the eighth day after operation. The survival periods of the 7 monkeys that received injections of oestrone (100 to 500 R.U. daily) varied from 18 to 216 days with an average of 69 days. During periods of oestrone treatment the hyperglycemia and glycosuria were usually although not invariably reduced, the loss of body weight was checked and the general condition of the animals was improved. Of the 7 totally depancreatized animals, 2 responded to treatment very well, 2 in a fair manner and 3 only slightly. Oestriol by mouth was without effect in 1 instance. In 2 cases the injection of a pituitary extract during periods of oestrone treatment resulted in an increased glycosuria.

The relation of the hypophysis and the adrenals to carbohydrate metab-

olism is considered and it is concluded that the most reasonable explanation for the effects observed with oestrone in these experiments is one based on the suppression of that pituitary activity which is directly or indirectly concerned with carbohydrate metabolism.

The writers take great pleasure in acknowledging the valuable services rendered in this investigation by Mr. Carl Pruette, who carried out most of the blood and urine sugar determinations.

REFERENCES

1. Houssay, B. A. and A. Biasotti: Endocrinology **15:** 511. 1931.
2. Barnes, B. O. and J. F. Regan: Endocrinology **17:** 522. 1933.
3. Collip, J. B.: J. Mt. Sinai Hospital **1:** 28. 1934.
4. Long, C. N. H. and F. D. W. Lukens: Proc. Soc. Exper. Biol. & Med. **32:** 326. 1934.
5. Hartman, F. A. and K. A. Brownell: Proc. Soc. Exper. Biol. & Med. **31:** 834. 1934.
6. Moore, C. R. and D. Price: Am. J. Anat. **50:** 13. 1932.
7. Nelson, W. O.: Endocrinology **18:** 33. 1934.
8. Nelson, W. O.: Endocrinology **19:** 187. 1935.
9. Barnes, B. O., J. F. Regan and W. O. Nelson: J.A.M.A. **101:** 926. 1933.
10. Shaffer, P. A. and M. Somogyi: J. Biol. Chem. **100:** 695. 1933.
11. Nelson, W. O. and M. D. Overholser: Proc. Soc. Exper. Biol. & Med. **32:** 150. 1934.
12. Nelson, W. O.: Proc. Soc. Exper. Biol. & Med. **32:** 452. 1934.
13. Nelson, W. O.: Physiol. Rev. In press, 1936.
14. Long, C. N. H. and F. D. W. Lukens: Proc. Soc. Exper. Biol. & Med. **32:** 743. 1935.
15. Barnes, B. O., V. B. Scott, H. W. Ferrill and J. M. Rogoff: Proc. Soc. Exper. Biol. & Med. **31:** 524. 1934.
16. Long, C. N. H.: Ann. Int. Med. **9:** 166. 1935.
17. Hohlweg, W.: Klin. Wnschr. **13:** 92. 1934.
18. Lane, C. E. Anat. Rec. **61:** 141. 1935.
19. Selye, H., J. B. Collip and D. L. Thomson: Proc. Soc. Exper. Biol. & Med. **32:** 1377, 1935.
20. Nelson, W. O.: Anat. Rec. **64:** 52. 1935.

THE EFFECT OF COMPLETE AND PARTIAL HYPOPHYSECTOMY IN ADULT ALBINO RATS ON NITROGEN, CALCIUM AND PHOSPHORUS METABOLISM

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The metabolic studies on hypophysectomized rats presented in this communication and similar studies on suprarenalectomized rats (1) previously reported were undertaken as part of an extended investigation of the factors concerned in the depression in natural resistance following hypophysectomy (2, 3, 4) and suprarenalectomy (5).

In an attempt to correlate the changes in natural resistance with metabolic disturbances in the animal, induced by such procedures as hypophysectomy and suprarenalectomy, the metabolism was studied in adult rats deprived of these glands. An analysis of the concentration of the chemical constituents of the blood yielded little information except in the terminal phases of either hypophyseal or suprarenal insufficiency. Metabolic studies are of greater value, as disturbances in the metabolism of various constituents are reflected earlier in changes in the retention of such substances than in abnormal variations in their concentration in the blood. The present study is concerned with the changes in the metabolism of nitrogen, calcium and phosphorus that follow hypophysectomy in the adult rat.

In an earlier paper the effect of suprarenalectomy in adult rats on the metabolism of nitrogen, sulfur, copper and iron has been reported (1). Urinary nitrogen excretion increases but the ability of the kidney to concentrate nitrogen is impaired. Urea excretion increases with little change in ammonia excretion. Uric acid remains unchanged. There is a pronounced creatinuria. Excretion of total urinary sulfur as well as of neutral sulfur increases. The percentage of sulfate excretion drops slightly. There is no change in copper and iron metabolism.

EXPERIMENTAL

Twenty-four male and female albino rats were used in the experiments. At the time of hypophysectomy they were about 2½ months of age. In 4 rats the posterior lobe and part of the anterior lobe only was removed. The rats were kept in groups of 4 in metabolism cages as described in a previous publication (6). Urine and feces were collected twice a week. The urine was analyzed for total nitrogen (Kjeldahl), creatine and creatinine (Folin's microchemical modification), calcium (volumetric), phosphorus (Fiske-Subbarow), and copper (7). Feces as well as food were analyzed for total nitrogen, calcium, phosphorus, copper and iron (8). The rats were studied for a control period of 9 weeks before and 12 weeks after hypophysectomy.

Nitrogen Metabolism. Urinary nitrogen excretion increased from 21 per cent of the nitrogen intake during the control period to 54 per cent during the first 3 weeks after operation and to 42 per cent during the following 9 weeks. In the group of partially hypophysectomized rats there was also an increase in the urinary nitrogen excretion, though less pronounced, from 26 per cent of the intake to 36 per cent during the first 3 weeks. After that the

TABLE 1. NITROGEN METABOLISM IN RATS BEFORE AND AFTER HYPOPHYSECTOMY. DAILY AVERAGE BY WEEKS.

Date	Urine	Total Nitrogen					Pref. Creatinine	Creatine
		Urine	Feces	Total Excretion	Intake	Retention		
	cc.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1934								
Nov. 16–22	1.5	65	122	187	367	180	3	1
Nov. 23–29	1.2	70	121	191	281	90	3	1
Nov. 30–Dec. 6	1.7	75	144	219	359	140	3	2
Dec. 7–13	1.6	91	129	220	348	128	3	2
Dec. 14–20	1.7	111	178	389	433	44	4	2
Dec. 21–27	1.5	82	150	232	407	175	3	1
1935								
Dec. 28–Jan. 3	2.3	52	143	195	370	175	4	2
Jan. 4–10	2.1	93	136	229	455	226	4	2
Jan. 11–17	2.0	92	134	226	411	185	4	1
Nov. 16–Jan. 17	1.7	81	140	221	381	160	3	2
*Jan. 23–28	14.2	144	64	208	223	15	2	6
Jan. 29–Feb. 4	10.1	122	100	222	267	45	2	6
Feb. 5–11	15.5	143	74	217	262	45	3	7
Jan. 23–Feb. 11	13.3	136	79	215	251	36	2	6
Feb. 12–18	15.0	135	135	270	319	49	2	3
Feb. 19–25	11.6	119	93	212	300	88	1	2
Feb. 26–Mar. 4	10.0	128	96	224	285	61	2	2
Mar. 5–11	12.0	151	123	274	367	93	2	3
Mar. 12–18	10.2	124	90	214	296	82	2	1
Mar. 19–25	6.0	129	79	208	308	100	3	1
Mar. 26–Apr. 1	3.6	133	107	240	363	123	3	1
Apr. 2–8	3.0	151	114	265	382	117	4	1
Apr. 9–15	5.0	168	134	302	378	76	4	2
Feb. 12–Apr. 15	8.5	138	108	246	333	87	2	2

* Hypophysectomized on January 18th.

nitrogen metabolism returned to normal and the urinary nitrogen excretion dropped to the initial value of 26 per cent of the intake, where it remained for the duration of the experiment. Creatinuria occurred during the first 3 weeks after operation in hypophysectomized male rats, as well as in males in which small amounts of hypophyseal tissue remained, but returned to normal in all cases. In the female rats no disturbance in creatine metabolism took place.

Calcium and Phosphorus Metabolism. There seems little doubt that the hypophysis is involved in the regulation of calcium metabolism, although on the low food intake which prevails after hypophysectomy paired-feeding con-

trols also showed a negative calcium balance. There was hardly any loss of calcium from the body but even when the intake was somewhat increased over that in the control period, when 12 per cent of the intake was retained, the balance remained around zero. In the partially hypophysectomized animals, on the other hand, the inability to utilize ingested calcium persisted for three weeks only. After that the balance became strongly positive when the calcium intake was increased. The principal increase in the calcium excretion was found in the feces, as is to be expected, since the urinary calcium excretion in rats is normally very small. But though the absolute value was small the excretion increased up to 600 per cent after hypophysectomy. In the partially hypophysectomized animals the increase amounted only to 100 to 200 per cent but it persisted even during the last period when the calcium balance became positive.

The phosphorus excretion increased progressively but the increase was restricted solely to the feces, the urinary excretion remaining practically constant, irrespective of the change in intake. The retention dropped from 27 per cent of the intake during the control period to 6 per cent but remained positive throughout. In the partially hypophysectomized group the retention returned to normal after the first 3 weeks following operation.

There were no significant changes in the copper and iron metabolism.

DISCUSSION

There is considerable evidence indicating that the anterior lobe of the hypophysis plays an important rôle in protein metabolism. The administration of the growth hormone in dogs is followed by a decrease in urinary nitrogen excretion (9) which may be as great as 50 per cent (10). A lessened urea excretion accounts for almost the entire decrease (10, 11). Following hypophysectomy in the dog the endogenous nitrogen metabolism is disturbed. Aschner (12) and Braier (13) observed a decrease in nitrogen excretion as compared to that of normal dogs. These findings are apparently inconsistent with those of Schaffer and Lee (14) and with our own observations. Schaffer and Lee studied changes in the nitrogenous constituents of the tissues themselves and of the whole carcass during short periods of treatment with growth hormone in normal and hypophysectomized rats. They noted a striking decrease in body protein in hypophysectomized rats which was roughly proportional to the length of time after hypophysectomy. They believe that this is not a secondary result of decreased food consumption but a catabolic effect. They reconcile their finding of an increased protein metabolism with Braier's conclusions of a decrease in endogenous protein metabolism in hypophysectomized dogs and toads by suggesting that immediately following hypophysectomy the increased rate of proteolytic processes may be greater than the diminution of the minimum endogenous catabolism, so that an increased endogenous catabolism may result. In the later period after hypophysectomy the former effect may subside, so that the decrease in endogenous catabolism predominates.

In our own experiments a marked increase in nitrogen excretion in the urine was observed in hypophysectomized rats. The nitrogen balance studies indicate a loss of nitrogen which is more than double the normal. This increase

persisted throughout the 3-months period of observation following the operation. These studies are consistent with the findings of Schaffer and Lee whose work suggested that the growth hormone is a specific stimulant of protein anabolism.

In a previous communication (1) we reported that suprarenalectomy in the rat is followed by a similar increased excretion of nitrogen in the urine.

TABLE 2. NITROGEN METABOLISM IN RATS BEFORE AND AFTER PARTIAL HYPOPHYSECTOMY. DAILY AVERAGE BY WEEKS.

Date	Urine	Total Nitrogen					Pref. Creatinine	Creatine
		Urine	Feces	Total Excret.	Intake	Retention		
	cc.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1934								
Nov. 20–26	1.1	82	149	231	352	121	3	2
Nov. 27–Dec. 3	1.1	75	127	202	237	35	3	2
Dec. 4–10	1.1	70	129	199	333	134	3	2
Dec. 11–17	1.4	82	130	212	307	95	3	2
Dec. 18–24	2.4	103	169	272	371	99	4	2
Dec. 25–31	1.3	82	145	227	370	143	3	2
1935								
Jan. 1–7	1.8	104	168	272	371	99	4	3
Nov. 20–Jan. 7	1.3	85	145	230	334	104	3	5
*Jan. 15–21	7.1	110	84	194	218	24	4	7
Jan. 22–28	6.4	111	119	230	305	75	2	6
Jan. 29–Feb. 4	4.7	82	129	211	317	106	2	1
Jan. 15–Feb. 4	6.1	101	111	212	280	68	3	2
Feb. 5–11	6.2	98	126	224	402	178	2	2
Feb. 12–18	5.4	115	138	253	339	86	2	2
Feb. 19–25	4.6	92	125	217	335	118	3	2
Feb. 26–Mar. 4	5.3	89	129	218	324	106	3	2
Mar. 5–11	5.8	106	129	235	363	128	3	3
Mar. 12–18	5.3	102	121	223	437	214	2	1
Mar. 19–25	4.3	80	106	186	359	173	3	2
Mar. 26–Apr. 1	5.0	76	89	165	277	112	3	1
Apr. 2–8	5.0	81	113	194	332	138	3	2
Feb. 5–Apr. 8	5.2	93	120	213	352	139	3	2

* Hypophysectomized on January 8th.

It may be that depression of the suprarenal cortical function induced by hypophysectomy (and withdrawal of the adrenotropic hormone) in part accounts for the increase in protein catabolism that follows removal of the hypophysis.

Removal of the hypophysis is associated with involutional changes in the thyroid with a marked drop in the basal metabolic rate. Though a decrease in oxygen consumption is not necessarily associated with decrease in protein catabolism it generally occurs. However, in the hypophysectomized rat other factors, at present not entirely clear, precipitate a destruction of protein in the tissues and rapidly overbalance any inhibitory effect exerted by diminished thyroid function. The depression in suprarenal cortical function and the withdrawal of the growth hormone of the anterior hypophysis contribute to the profound effect on protein catabolism induced by hypophysectomy in the rat.

Creatinuria occurs in the male rats during the first 3 weeks after operation. The significance of this finding is not entirely clear. Several explanations may be advanced for the creatinuria which these animals show. It may be secondary to an increased protein catabolism. This is suggested by the experiments of Terroine and his associates (15) who observed creatinuria in normal pigs, in which an increased endogenous protein catabolism was induced by a high

TABLE 3. CALCIUM AND PHOSPHORUS METABOLISM BEFORE AND AFTER HYPOPHYSECTOMY. DAILY AVERAGE BY WEEKS.

Date	Calcium				Phosphorus			
	Excretion		Intake	Retention	Excretion		Intake	Retention
	Urine	Feces			Urine	Feces		
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1934								
Nov. 16–22	0.3	109	131	22.6	2.8	62.5	88.9	23.6
Nov. 23–29	0.3	90	101	10.6	2.8	60.3	88.4	25.3
Nov. 30–Dec. 6	0.3	125	129	3.9	2.0	61.5	87.3	23.8
Dec. 7–13	0.3	120	125	4.5	2.5	62.0	84.6	20.1
Dec. 14–20	0.4	131	146	14.8	2.0	75.3	98.8	21.5
Dec. 21–27	0.3	119	146	26.8	3.5	66.9	99.0	28.6
1935								
Dec. 28–Jan. 3	0.3	131	146	14.9	2.0	75.3	98.7	21.4
Jan. 4–10	0.4	128	157	28.7	3.0	70.9	110.7	36.8
Jan. 11–17	0.4	133	161	27.5	2.8	72.9	104.3	28.5
Nov. 16–Jan. 17	0.3	121	138	17.2	2.6	67.5	95.6	25.5
*Jan. 23–28	1.3	73	80	5.7	2.8	40.4	54.3	11.1
Jan. 29–Feb. 4	1.3	126	127	−0.2	1.6	67.0	69.7	1.1
Feb. 5–11	1.0	118	125	6.3	2.0	54.8	67.1	10.3
Jan. 23-Feb. 11	1.2	108	112	2.5	2.1	54.8	64.2	7.3
Feb. 12–18	2.0	153	152	−2.4	2.3	81.5	82.0	−1.8
Feb. 19–25	1.7	141	143	0.5	3.4	73.5	77.0	0.1
Feb. 26–Mar. 4	1.7	150	136	−15.6	2.9	71.3	73.0	−1.2
Mar. 5–11	2.1	175	175	−2.1	3.8	90.7	94.0	−0.5
Mar. 12–18	1.1	125	141	15.5	1.2	71.1	76.0	3.7
Mar. 19–25	1.2	132	148	14.3	1.7	63.0	79.0	14.3
Mar. 26–Apr. 1	1.9	167	173	4.4	1.0	79.7	93.0	12.3
Apr. 2–8	1.9	169	182	11.2	1.0	85.5	98.0	11.5
Apr. 9–15	2.4	173	180	4.9	1.1	87.0	97.0	8.9
Feb. 12–Apr. 15	1.8	154	159	3.4	2.0	78.1	85.4	5.3

* Hypophysectomized on January 18th.

carbohydrate diet to which a large amount of acid had been added. On the other hand the creatinuria may be associated with a disturbance in carbohydrate metabolism. Brentano (16) observed a close correlation between creatinuria and glycogen content of skeletal muscles regardless of the procedure employed to induce decreases in muscle glycogen. The degree of creatinuria is said to depend not so much upon the absolute amount of glycogen in the muscles as upon the extent and rapidity with which it is reduced (17). Houssay and his co-workers (18) found that the liver glycogen in hypophysectomized toads diminishes rapidly and is maintained at a low level for about 3 weeks after operation. In rats also a compensatory mechanism develops after 3

weeks which permits the creatine metabolism to return to normal. Neither of these suppositions, however, accounts for the sex difference as regards creatinuria in hypophysectomized rats. Marked differences in creatine output in male and female human subjects have been observed. Creatinuria is frequent in children and Beumer and Fasold (19) express the opinion that sexual maturity is a significant factor in bringing about the destruction of creatine.

TABLE 4. CALCIUM AND PHOSPHORUS METABOLISM BEFORE AND AFTER PARTIAL HYPOPHYSECTOMY. DAILY AVERAGE BY WEEKS.

Date	Calcium				Phosphorus			
	Excretion		Intake	Retention	Excretion		Intake	Retention
	Urine	Feces			Urine	Feces		
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1934								
Nov. 20–26	0.3	115	126	11.4	2.8	65.5	85.5	17.2
Nov. 27–Dec. 3	0.3	94	85	−9.4	2.5	53.9	57.6	1.2
Dec. 4–10	0.3	93	120	26.9	2.3	59.0	81.0	19.7
Dec. 11–17	0.3	108	111	2.7	2.6	59.7	74.7	12.4
Dec. 18–24	0.4	117	136	18.8	2.1	67.6	90.4	20.7
Dec. 25–31	0.4	110	133	22.3	3.1	61.2	90.0	25.7
1935								
Jan. 1–7	0.3	117	136	18.8	2.1	67.6	90.4	20.7
Nov. 20–Jan. 7	0.3	108	121	13.1	2.5	62.1	81.4	16.8
*Jan. 15–21	0.9	84	78	−7.0	2.6	46.4	53.0	4.0
Jan. 22–28	1.1	112	110	−3.3	1.9	57.2	74.2	15.1
Jan. 29–Feb. 4	0.6	143	151	7.8	0.7	73.3	81.3	7.3
Jan. 15–Feb. 4	0.9	113	113	−0.9	1.7	59.0	69.5	8.8
Feb. 5–11	0.7	149	192	42.3	1.4	75.0	103.0	26.6
Feb. 12–18	1.1	152	162	9.1	2.0	76.8	87.0	8.2
Feb. 19–25	0.5	156	160	3.1	1.5	81.7	86.0	2.8
Feb. 26–Mar. 4	0.5	145	154	8.5	1.4	66.1	83.0	15.5
Mar. 5–11	0.6	152	173	20.4	1.0	76.1	93.0	15.9
Mar. 12–18	0.3	138	208	69.7	1.6	70.4	112.0	40.0
Mar. 19–25	0.5	122	171	58.3	0.8	68.3	92.0	22.9
Mar. 26–Apr. 1	0.4	117	145	27.6	1.2	56.5	78.0	20.3
Apr. 2–8	0.5	143	158	14.8	1.3	72.4	85.0	11.3
Feb. 5–Apr. 8	0.6	142	169	27.0	1.4	71.5	91.0	18.1

* Hypophysectomized on January 8th.

Buadze (20) finds that boys excrete creatine until 6 to 10 years of age, whereas girls manifest a constant creatinuria until puberty, after which a cyclic creatinuria is said to occur. The author believes it probable that the male sex hormone inhibits the output of creatine and that the female hormone promotes its appearance. Remen (21) reports that after intravenous injection of creatine into normal men practically none of the substance appears in the urine but that a pronounced creatinuria occurs in the aged under similar conditions. Emasculation sometimes leads to a continuation into adult life of the prepubertal creatinuria. Apparently such a relationship between sex gland activity and creatine metabolism does not occur in the rabbit (22). It would seem likely, therefore, that the creatinuria in the male hypophysectomized rat is secondary to the atrophy of the testes. This, however, does not explain

its transient character. The creatinuria in suprarenalectomized rats is of equal severity in male and female rats and is persistent (1). In these animals it is probably associated with glycogen depletion and increased protein catabolism.

A relationship between the hypophysis and calcium metabolism is indicated by the effects of hypophysectomy and the administration of anterior lobe extracts on the growth and ossification of the bones in animals and by the striking skeletal changes in acromegaly and hypophyseal gigantism in man. Calcium may be retained in acromegalics (23). The serum calcium may be high (24) or low (25) depending on the degree of disturbance of the basal metabolism. Houssay and Mazzocco (26) observed a drop of 11 per cent in the serum calcium of hypophysectomized dogs, though Mazzocco (27) and Gerschman (28) found no effect on the concentration of plasma calcium. The growth of teeth is impaired in hypophysectomized dogs (29) and the epiphyses remain open longer in the operated than in the control dogs. The fall in serum calcium of young hypophysectomized dogs, however, is not constant.

The persistent administration of aqueous extracts of ox anterior hypophysis over a period of 14 months in dogs resulted in the production of experimental acromegaly and the serum calcium increased about 11 per cent (30). On the other hand Hogben and Charles (31) observed a fall in serum calcium in female normal and ovariectomized rabbits repeatedly injected with fresh saline emulsions of ox hypophysis.

Removal of either the anterior lobe alone or of both lobes in Xenopus laevis caused a persistent fall in serum calcium of 37 and 26 per cent respectively (32). Shapiro and Zwarenstein (33) found that the injection of 'Antuitrin' into completely hypophysectomized animals caused a rise, and injection of pituitrin, a fall in serum calcium. These observations strongly suggest a relationship of the hypophysis to calcium metabolism.

Pugsley and Anderson (34) state that hypophysectomized rats on a low calcium diet show a negative calcium balance which becomes positive on administration of growth hormone but they give no details as regards the amount of calcium fed. In the experiments reported in the present communication the disturbance in calcium metabolism in hypophysectomized rats was not as striking but was definite. On a low calcium intake paired-feeding controls also showed a negative calcium balance. On an increased intake the inability to utilize ingested calcium persisted in the hypophysectomized rats. The principal increase in the calcium excretion was found in the feces.

It is possible that the disturbance in calcium metabolism may be secondary to the effect of hypophysectomy on the gonads, since Shapiro and Zwarenstein observed a persistent fall in the calcium content of the serum after gonadectomy. However, in their experiments on animals in which the pars tuberalis had regenerated but where the ovaries remained completely involuted, the serum calcium rose to normal. It is more likely that the hypophysis controls calcium metabolism through the parathyroids.

SUMMARY

The nitrogen, calcium, phosphorus, copper and iron metabolism of completely and partially hypophysectomized rats was studied. Urinary nitrogen ex-

cretion was more than doubled during the first 3 weeks after operation and the increased nitrogen excretion persisted during the following 9 weeks. The disturbance in nitrogen balance was transient and less pronounced in those rats in which the posterior lobe and only part of the anterior lobe had been removed. Creatinuria occurred during the first 3 weeks after operation in male rats either completely or partially hypophysectomized but returned to normal in all cases. No disturbance in creatine metabolism occurred in these females.

The calcium excretion increased and even on a moderately high calcium intake the balance remained around zero. The principal increase in the calcium excretion was found in the feces. The calcium excretion in the urine, though slight, was increased several hundred per cent. In the partially hypophysectomized rats the disturbance in calcium utilization persisted for a period of only 3 weeks. The phosphorus excretion in the feces increased progressively, the urinary excretion remained constant. There were no significant changes in the copper or iron metabolism.

REFERENCES

1. Sandberg, M., and D. Perla: J. Biol. Chem. **113:** 35. 1936.
2. Perla, D., and S. H. Rosen: Arch. Path. **20:** 222. 1935.
3. Perla, D.: Proc. Soc. Exper. Biol. & Med. **32:** 655. 1935.
4. Perla, D.: Proc. Soc. Exper. Biol. & Med. **33:** 121. 1935.
5. Perla, D., and J. Marmorston: Arch. Path. **16:** 379. 1933.
6. Sandberg, M., and D. Perla: J. Exper. Med. **60:** 395. 1934.
7. McFarlane, W. D.: Biochem. J. **26:** 1022. 1932.
8. Elvehjem, C. A., and E. B. Hart: J. Biol. Chem. **67:** 43. 1926.
9. Teel, H. M., and H. Cushing: Endocrinology **14:** 157. 1930.
10. Gaebler, O. H.: J. Exper. Med. **57:** 349. 1933.
11. Lee, M. O., and N. K. Schaffer: J. Nutrition **7:** 337. 1934.
12. Aschner, B.: Arch. f. d. ges. Physiol. **146:** 1. 1912.
13. Braier, B.: Rev. Soc. argent. de biol. **7:** 140; 254; 340. 1931.
14. Schaffer, N. K., and M. Lee: J. Biol. Chem. **108:** 355. 1935.
15. Terroine, E. F., and M. Champagne: Bull. Soc. chim. biol. **15:** 235. 1935.
16. Brentano, C.: Arch. f. exper. Path. u. Pharmakol. **155:** 21. 1930.
17. Brentano, C.: Arch. f. exper. Path. u. Pharmakol, **163:** 156. 1931-32.
18. Houssay, B. A., E. di Benedetto and P. Mazzocco: Compt. rend. Soc. de biol. **113:** 465. 1933.
19. Beumer, H., and H. Fasold: Klin. Wchnschr. **10:** 937. 1931.
20. Buadze, S.: Ztschr. f. d. ges. exper. Med. **90:** 763. 1933.
21. Remen, L., Ztschr. f. d. ges. exper. Med. **80:** 238. 1931.
22. Schrire, J., and H. Zwarenstein: Biochem. J. **26:** 118; 1886. 1932. **27:** 1337, 1933. **28:** 356. 1934.
23. Medigreceanu, F., and L. Kristeller: J. Biol. Chem. **9:** 109. 1911.
24. Mirvish, L., and H. A. Shapiro: 1933, cited by Zwarenstein, H., Biol. Rev. **9:** 299. 1934.
25. Waldorp, C. P.: Compt. rend. Soc. de biol. **94:** 492. 1926.
26. Houssay, B. A., and P. Mazzocco: Compt. rend. Soc. de biol. **86:** 409. 1922.
27. Mazzocco, P.: Compt. rend. Soc. de biol. **97:** 594. 1927.
28. Gerschman, R.: Compt. rend. Soc. de biol. **108:** 494. 1931.
29. Koster, S., and A. Geesink: Arch. f. d. ges. Physiol. **222:** 293. 1929.
30. Putnam, T. J., E. B. Benedict and H. M. Teel: Arch. Surg. **18:** 1708. 1929.
31. Hogben, L. T., and E. Charles: J. Exper. Biol. **9:** 139. 1932.
32. Charles, E.: Proc. Roy. Soc. **107:** 504. 1931.
33. Shapiro, H. A., and H. Zwarenstein: J. Exper. Biol. **10:** 186. 1933.
34. Pugsley, L. J., and E. M. Anderson: Am. J. Physiol. **109:** 85. 1934.

THE COMPOSITION OF WEIGHT LOST AND THE NITROGEN PARTITION OF TISSUES IN RATS AFTER HYPOPHYSECTOMY[1]

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Ablation of the anterior pituitary gland in rats results not only in growth stasis but commmonly in a marked loss of body weight. In adult animals on their growth plateaus, if ablation is complete and no replacement therapy is instituted, there occurs an initial sharp fall for a period of a few days, followed by a further progressive slow loss. Such adult animals 2 months after operation commonly show a loss of 20 to 40 per cent of their initial weight. Rats hypophysectomized before their growth plateaus have been reached, especially those weighing 80 to 120 grams, lose a smaller proportion of their original weight. They commonly remain on a plateau after this initial loss and never regain their former weight level. In still younger hypophysectomized rats, up to 80 grams or so, there may be only a slight initial loss in weight, followed by a gradual return and maintenance of the weight close to the original level reached before hypophysectomy.

As might be expected, there is a marked decrease in food consumption after hypophysectomy. In the present paper the relationship of this low food consumption to the loss in body weight has been determined by restricting the food eaten by control rats to the same amounts taken voluntarily by their hypophysectomized mates .The character of the weight lost in the two groups has also been compared by means of carcass analysis and the levels of a number of tissue non-protein nitrogenous constituents at various times after hypophysectomy have been determined.

EXPERIMENTAL PROCEDURES

Pairs or trios of litter-mate rats of the same sex and initial weight were selected. After a week's trial on paired feeding, one rat of each pair or trio was hypophysectomized. Care was taken to achieve complete ablation and in case of doubt the animal was discarded from the series. The control member of each pair was subjected to a sham operation of the same sort except that the pituitary was not disturbed. The third animal of each trio, designated as a 'check' was killed on the day the operation was made. The check rats were analyzed and their average composition was taken to be that of their control and hypophysectomized mates at the beginning of the experiment.

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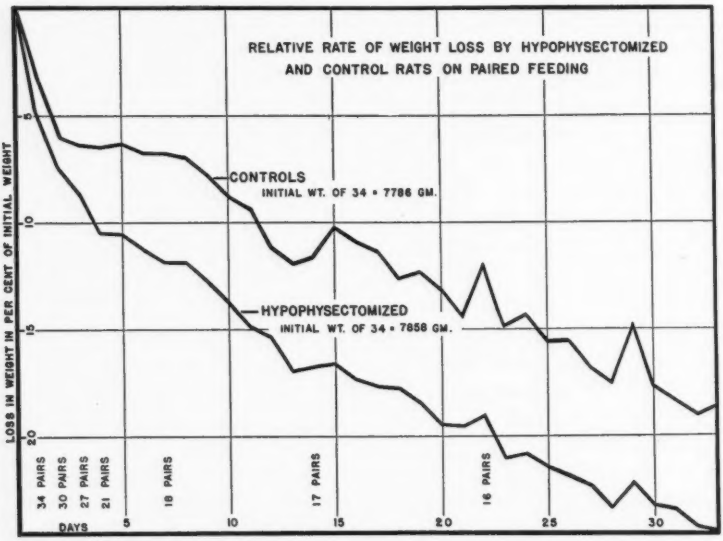
The control and hypophysectomized mates of each pair, after operation, were continued on paired feeding (1). The two animals of a pair had their daily and weekly food allowances equalized to within 50 mg. The amount of food given was determined by the less hungry member of the pair, the hypophysectomized rat in every case. The length of the experiment varied from 24 hours to 33 days with various groups of animals. At various times all of the rats of a given series were killed and their carcasses analyzed according to methods described elsewhere (2).

RESULTS

The hypophysectomized rats showed the usual and expected sequellae of pituitary removal (3). In addition their food consumption was markedly de-



creased, although it varied considerably from day to day (from 3 to 10 grams). For a group of 16 hypophysectomized females which were followed for 33 days it averaged 6.2 grams per rat per day. Ad libitum fed normal rats of the same age consume about twice this amount. The control rat of each pair invariably ate its entire daily ration soon after feeding. When killed, the contents of the alimentary tracts averaged 2.6 per cent of the live body weight for the controls and 2.9 per cent for the hypophysectomized.

Rate of Weight Loss. During the first few days after the operation the hypophysectomized rats lost weight at a more rapid rate than their control mates on the same food consumption (see figure). Thus 24 hours after the operation the controls had lost 3.4 per cent and the hypophysectomized 4.8 per cent of their initial weight. Four days after operation the losses were: controls 6.5 per cent; hypophysectomized 10.5 per cent.

After the first week the rate of weight loss in the two groups was about

the same. It is noteworthy that in several instances when the group weight of the hypophysectomized rats happened to rise for a day or so above the trend-line (due to chance coincidences of individually increased food allowances) the control groups showed much sharper increases, although they returned to about the same trend-line. In the series that was continued for 33 days the 16 controls had lost 18.6 per cent of their initial weight while their 16 hypophysectomized mates had lost 24.4 per cent.

Composition of the Weight Lost. The composition of the control and hypophysectomized rats and the composition of the weight lost by each group, as shown in table 1, offers more particular evidence. The most striking differ-

TABLE 1. COMPOSITION OF CONTROL AND HYPOPHYSECTOMIZED RATS AT BEGINNING AND END OF 33 DAYS PAIRED-FEEDING PERIOD, AND COMPOSITION OF THE WEIGHT LOST.

	Live Weight of Group	Empty Carcass Weight	Composition, by Weight and Percentage, of Empty Carcass and of Loss in Empty Carcass Weight								Heat Value of Empty Carcass
			Water		Ether Extract		Fat-free Dry Tissue		Nitrogen		
	gm.	gm.	gm.	%	gm.	%	gm.	%	gm.	%	Cal.
16 Controls, Beginning	3404	3318[1]	2124[1]	64.0	435[1]	13.1	759[1]	22.9	94.5[1]	2.85	7450[1]
16 Hypophysectomized, Beginning	3341	3260[1]	2086[1]		427[1]		747[1]		92.8[1]		7340[1]
16 Controls, End[2]	2772	2710	1775	65.5	173	6.4	762	28.1	95.6	3.53	5055
16 Hypophysectomized, End[2]	2527	2420	1504	62.2	306	12.6	610	25.2	75.5	3.12	5621
Loss by 16 Controls	632	608	349	57.5	262	43.1	−3		−1.1		2395
Loss by 16 Hypophysectomized	814	840	582	69.3	121	14.4	137	16.3	17.3	2.06	1719

[1] Estimated from composition of litter-mate check rats.
[2] After 33 days paired-feeding.

ences in the two groups were in fat and nitrogen content. The control rats on the sub-maintenance level of food consumption lost 60 per cent or 262 grams of their initial fat content but retained their original amounts of nitrogen and of fat-free dry tissue. The level of food consumption was apparently sufficiently low to cause a marked depletion of body fat stores but not so low as to necessitate utilization of body protein for energy purposes. The diet fed contained approximately 20 per cent protein and 6 per cent fat.

The hypophysectomized pair-fed mates, on the other hand, utilized only 121 grams or 28.3 per cent of their initial fat stores, but lost 17.3 grams or 18.6 per cent of their original nitrogen content. In percentage of empty carcass weight their value for fat was nearly the same at the beginning and end of the experiment, while the value for the controls was halved at the end.

The changes in water content were not highly significant but were in the direction that would be expected. The differences in fat-free dry tissue and in energy values of the carcasses reflect the differences in nitrogen and fat content.

The control rats derived 17 per cent of their average daily energy ex-

penditure from their own body fat, while their hypophysectomized mates derived 8 per cent from their own fat stores and 4.4 per cent from body stores other than fat.

As shown by the data in table 2, the total energy metabolism was practically the same in the two groups. In both it was about 23 per cent lower than the value which would be expected in normal rats of similar age given food ad libitum (4). The values in table 2 have a reasonably high degree of accuracy despite the fact that the initial composition of the rats was necessarily estimated from analyses of litter mates. An error of 10 per cent in the estimated initial fat content, for example, would change the calculation of total energy metabolism by about 2 per cent.

TABLE 2. ENERGY METABOLISM OF CONTROL AND HYPOPHYSECTOMIZED FEMALE RATS. PAIRED FEEDING FOR 33 DAYS.

	Mean of Summed Daily Body Wt. Gm/rat	Food Eaten Gm./rat/day	Metab. Energy of Food Cal./rat/day	Energy Lost from Carcass Cal./rat/day	Total Energy Metabolism	
					Cal. day/100 Gm. B.W.	Cal./day/Sq. M. B.S.
14 Controls	184	6.21	22.3	4.6*	14.6	939
14 Hypophysectomized	170	6.20	21.7	3.2**	14.7	914

* All from body fat stores.
** 2.0 Cal./rat/day from body fat and 1.1 Cal./rat/day from body stores other than fat.

The foregoing data indicate that hypophysectomized rats mobilize and use a relatively large proportion of their original reserves of body protein and use a relatively small portion of their body fat stores, as compared with normal controls restricted to the same low level of food consumption. Two explanations suggest themselves: a) The hypophysectomized rat may suffer from a relative inability to mobilize and use stored body fat and consequently is forced to draw more upon body protein to supply energy demands; b) The animal may suffer from a relative inability to retain and conserve its body protein reserves and in consequence of a relatively increased mobilization of protein has a lessened demand upon body fat for energy purposes. Both of these conditions may, of course, exist simultaneously.

There is obviously no absolute inability on the part of the hypophysectomized rat to utilize its stored body fat or to burn ingested fat. The 16 hypophysectomized animals of table 1 utilized 121 grams of their body fat stores and burned 190 grams of ingested fat in the 33 day period. Despite their 24 per cent loss in body weight the percentage of fat at the end of that period was nearly the same as at the beginning. This is in marked contrast to the control animal whose food allowance is restricted to the same sub-maintenance level as that voluntarily assumed by its hypophysectomized mate.

The data of several other experiments offer further evidence on these points. Six hypophysectomized rats were kept without food for periods of 6 to 14 days after operation and analyzed. This group lost 26 per cent of their initial body weight, 40 per cent of their body fat and 10 per cent of their original nitrogen content. Controls without food for approximately the same

periods lost 24 per cent of their body weight, 65 per cent of their body fat and 4 per cent of their original nitrogen.

Three hypophysectomized females were given a high fat (64%) high protein (25%) diet, ad libitum, for 14 days after operation. They lost only 5.5 per cent of their initial body weight. Carcass analysis showed they had gained 31 grams of body fat or 57 per cent of their initial amount, but had lost 9 per cent of their initial nitrogen content.

Five hypophysectomized rats were placed on a fat-free, high protein (26%), high carbohydrate diet for 14 days after operation and then analyzed. The amount of food fed was limited so that the caloric intake was about one-half that which hypophysectomized rats voluntarily consume. At the end of

TABLE 3. RATIOS, LIVER WEIGHT TO BODY WEIGHT IN CONTROL AND HYPOPHYSECTOMIZED RATS ON PAIRED FEEDING.

	Average Body Wt. Gm.	Liver Wt. Mean and σ_m Gm.	Liver Wt./100 Gm. B.W. Mean and σ_m
23 Controls	224	7.0±0.21	3.13±0.06
27 Hypophysectomized	214	5.8±0.14	2.72±0.06
Difference	10	1.2±0.25	0.40±0.08

14 days these rats had lost 21 per cent of their initial body weight, 56 per cent of their original fat stores and an insignificant amount of body nitrogen.

It is thus apparent that the hypophysectomized rat is able to deposit body fat from ingested food and is able to utilize both ingested fat and its own fat stores if placed under proper conditions to necessitate such utilization. Soskin (5) has presented evidence that the hypophysectomized fasting dog is unable to replenish its depleted blood sugar from ingested fat, whereas the normal fasting dog can do so. This suggests that the effective demand upon fat metabolism is actually less in the hypophysectomized animal than in the control.

Liver Weight. After hypophysectomy in a rat its liver undergoes considerable atrophy, as shown by the data in table 3. The ratio of liver weight to body weight was also less in these 27 hypophysectomized rats than in their control mates limited to the same food consumption. These animals were killed in pairs at various periods of time, from 24 hours to 33 days, after operation. In 12 other pairs of rats kept for 33 days, the ratios were 3.26 for the controls (paired feeding) and 2.57 for the hypophysectomized. In our normal rats with food ad libitum the value of the ratio is usually above 3.5.

Nitrogen Partition of Tissues. The nitrogen partition of the liver and of the empty carcass minus the liver was determined in groups of hypophysectomized rats and their litter-mate, pair-fed controls at intervals of 24 hours to 33 days after operation. The results for non-protein nitrogen, amino acids and urea are given in table 4. The length of time after hypophysectomy (24 hours to 33 days) was found to make but little difference in the levels of the several constituents, so all of the groups of hypophysectomized rats were averaged and all of the groups of controls were treated together. In several in-

stances 2 hypophysectomized rats were paired with a single litter-mate control so that in case an ablation was incomplete or the animal died a pair would still be available.

There were significant increases in the liver of hypophysectomized rats in amino acid, urea and total non-protein nitrogen. The whole body tissue without the liver showed similar tendencies but the differences were not significant statistically except possibly for amino acids. The qualitative differences were consistent in all of the 7 groups into which the animals were divided and which were killed at intervals of 1, 2, 3, 3, 5, 14 and 33 days. Normal control litter mates receiving food ad libitum and gaining in body weight had about the same levels of amino acids and N.P.N. as the hypophysectomized rats but urea concentrations were usually lower, both in liver and whole carcass.

TABLE 4. SUMMARY OF NITROGEN PARTITION IN TISSUES OF CONTROL AND HYPOPHYSECTOMIZED RATS. VALUES IN MG. PER 100 GM. FRESH TISSUE. PAIRED-FEEDING.

	Total N.P.N.		Amino Acid N		Urea N	
	Mean and σ_m	S.D. of Distribution	Mean and σ_m	S.D. of Distribution	Mean and σ_m	S.D. of Distribution
			A. Liver			
23 Controls	144±3.1	±14.6	47.1±1.02	±4.90	11.0±0.79	±3.70
27 Hypophysectomized	162±2.0	±10.3	51.4±0.50	±2.73	17.8±1.02	±5.31
Difference	18±3.7		4.3±1.2		6.8±1.29	
			B. Empty Carcass Without Liver			
23 Controls	224±4.9	±23.7	42.7±0.70	±3.38	27.2±0.82	±3.92
27 Hypophysectomized	234±4.3	±22.3	45.4±0.59	±3.07	28.9±0.82	±4.26
Difference	10±6.5		2.7±0.92		1.7±1.16	

Ammonia, uric acid, sulfur, cystine, creatine (carcass), creatinine (carcass), glutathione, albumin and globulin were also determined. None of these constituents showed consistent or significant differences in concentration between the control and hypophysectomized groups. The concentration of glutathione did tend to be lower in both liver and carcass of the hypophysectomized rats but the variability of this constituent was so great that no significant difference could be established.

The changes in tissue amino acids and urea were not great but were in the direction which would be expected if endogenous protein catabolism is increased in hypophysectomized animals whose protein intake in their food is barely that which suffices for maintenance of nitrogen balance in normal controls. The changes are the opposite of those previously reported in rats treated with growth hormone (6).

While such studies of the concentration of various nitrogenous constituents in the tissues are essential in giving a complete picture of the rôle of the pituitary in protein metabolism, it is considered that metabolic and carcass analysis determinations offer more promise in depicting the end results of

disturbances in the homeostatic mechanisms which regulate the levels of the several constituents in the blood and tissues. Such procedures reveal the cumulative effects of these disturbances which may be only slightly reflected in the concentrations of tissue constituents at any given time.

SUMMARY

The loss in body weight over a period of 1 to 33 days was 20 per cent greater in 34 hypophysectomized rats than in their litter-mate controls restricted to the same low food intake as that voluntarily consumed by the hypophysectomized rats. Carcass analysis of 16 pair-fed controls showed that they lost 60 per cent of their initial fat content in 33 days but retained all of their original body nitrogen. Their 16 hypophysectomized mates lost only 28 per cent of their initial fat stores but 19 per cent of their original nitrogen content. The total energy metabolism was practically the same in the two groups but was 23 per cent less than normal controls fed ad libitum. Atrophy of the liver after hypophysectomy was greater proportionately than the loss in body weight. Determination of the nitrogen partition of tissues showed that 27 hypophysectomized rats had significantly higher levels of amino acid, urea and total non-protein nitrogen in their livers than 23 pair-fed controls. Similar but smaller differences in these constituents obtained for the whole carcass. Other tissue nitrogenous constituents were not consistently or significantly affected.

REFERENCES

1. Mitchell, H. H., and J. R. Beadles: J. Nutrition **2:** 225. 1930.
2. Ayres, G. B., and M. Lee: J. Biol. Chem. In press.
3. Smith, P. E.: Am. J. Anat. **45:** 205. 1930.
4. Lee, M. O., and N. K. Schaffer: J. Nutrition **7:** 337. 1934.
5. Soskin, S., I. A. Mirsky, L. M. Zimmerman and N. Crohn: Am. J. Physiol. **114:** 110. 1935.
6. Schaffer, N. K., and M. Lee: J. Biol. Chem. **108:** 355. 1935.

THE EFFECT OF HYPOPHYSECTOMY AND OF PHYONE INJECTIONS ON THE PANCREAS AND LIVER OF THE NEWT

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Observations on the histology of the pancreatic islands of animals in hyperpituitary or hypopituitary states are rare in contrast to the abundance of physiological data. Anselmino and his co-workers (1, 2, 3) have described an increase in number and size and a new formation of islands in rats after 6 to 7 injections of an anterior lobe extract. Aron (4) has induced a premature appearance of islands in foetal guinea pigs injected in utero with an anterior lobe preparation. Kojima (5) discovered an accumulation of granules in the island cells of rats after posterior lobe feeding but none after anterior lobe feeding. Reports of negative or doubtful findings predominate, such as those of Putnam et al (6) and Evans et al (7) who found pancreases much larger than normal but no increase or histological differences in islet tissue of dogs made acromegalic by long-continued injections of anterior lobe extracts. Lucke (8) also observed no histological changes in islands of rats whose carbohydrate metabolism had been modified by injections of anterior pituitary extracts. In protocols of supposed pituitary basophilism (9) there is recorded in one case a "questionable enlargement of the pancreatic islets," in another "an unusual number of islets," and in another "the pancreatic islets were relatively few and atrophic in the part of the gland that had escaped necrosis." Though generalized splanchnomegaly occurs in acromegaly, histological data on the islands are scarce and their functional derangement alone is apt to be reported. In one case (10) the islands were more numerous and some were larger than normal, measuring 600μ in diameter whereas 200μ is considered normal and 400μ markedly hypertrophic and in 4 others (11), 1 with diabetes had the largest islets some measuring more than 400μ, while in the other 3 (1 diabetic and 2 non-diabetic) they averaged 220, 200, and 180μ.

Either the pancreas is not mentioned in papers on hypophysectomy (12, 13, 14) or its weight and size decrease is recorded, accompanied by the statement that determination of island changes was not attempted (15) or that no pancreatic lesions were found (16).

Because animals used mainly in work on the pancreas and pituitary (guinea pigs, dogs, rats, rabbits, cats) have such large pancreases that it is impracticable to study the entire gland in serial sections and determine definitely the island number, it was thought that an investigation of the urodele pancreas whose size makes serial sections and island counts feasible might furnish definite data as to the pancreatic islands in normal, hypopituitary and hyperpituitary states. Since the liver and its glycogen appear to vary also in these conditions in the vertebrates, it also was examined for changes that might correlate with pancreatic ones.

MATERIAL AND METHODS

Sixty adult male newts (Triturus viridescens) were divided into groups of 15 each: untreated normals; phyone-injected normals; untreated hypophysectomized; phyone-injected hypophysectomized. The sella contents and neighboring brain tissues were sectioned to determine the completeness of hypophysectomy. In 1 animal hypophysectomized for 2 weeks, a sizeable fragment of pars anterior was found. The data on this animal were not used in the computations except in the determination of liver glycogen. Treated animals were injected intraperitoneally for 14 days with 0.2 cc. of phyone[1] diluted 1:9 with 0.4 per cent NaCl. Untreated hypophysectomized animals were killed on the 17th day following the operation, while the phyone-injected hypophysectomized group was killed 16 days after the operation. Injections in the latter group were started 2 days after hypophysectomy to allow partial recovery from the operation.

Ten pancreases from each group were fixed in Helly's fluid, sectioned longitudinally at 10μ and stained in Delafield's haematoxylin and eosin. These serial sections were studied to determine the number of islands, the number of sections through which each island ran and the total number of sections of the pancreas. In this way a rough ratio between island and total pancreatic tissue was obtained. The other 5 glands in each group were fixed according to Bowie's method (18), cut longitudinally at 4μ, stained in Biebrich scarlet and neutral ethyl violet and examined for structure of the island cells. At killing the animals were weighed but the pancreases were brought to 70 per cent alcohol before weighing.

A comparison of the size of island nuclei was made in the groups by projecting ($\times$1000) 10 nuclei from an island in each pancreas (59 in all) and measuring them. Only 1 island from each gland was considered since the island nuclei do not vary greatly in size within a single pancreas. The nuclei were measured in 2 diameters and the average taken.

The tip end of the central and longest lobe of the liver was fixed in every case in Zieglwallner's fluid (19) which preserves both glycogen and fat. This was cut at 5μ and stained with Best's carmine for glycogen. The rest of the liver was weighed while fresh and the glycogen extracted by boiling and precipitation with 95 per cent alcohol. The alcohol was evaporated and the glycogen weighed. This method of glycogen extraction gives an approximate estimation of the relative glycogen content of the livers in the 4 groups.

The animals were kept in a cool room (about 65°F.) in jars of tap water changed daily. They were fed pieces of hamburger steak twice during the experiment, one week after operation and the day before killing. The week's fast was imposed to allow recovery from the operation and if possible to bring the glycogen stores to the same general level in all.

OBSERVATIONS

Pancreas. An examination of the data in table 1 shows that in the hyperpituitary newts the pancreas weight increased somewhat while in the hypo-

[1] The phyone used was the Van Dyke and Wallen-Lawrence preparation (17) and was secured from the Wilson Laboratories, Chicago.

pituitary newts it decreased slightly, although neither change was significant statistically. In total number of islets and number per milligram of pancreatic tissue there was a significant increase in both groups. Increase of island tissue was also evident in the section ratios. It was common to find many small islands located near the ducts and running through 2 or 3 sections in addition to the 2 or 3 large ones, appearing in 20 to 30 sections, and medium-sized ones, running through 2 to 15 sections, characteristic of the normal pancreas. Furthermore the size of the island nuclei decreased significantly in both groups.

TABLE 1. DATA ON HYPOPHYSECTOMIZED AND NORMAL NEWTS AND HYPOPHYSECTOMIZED NEWTS INJECTED FOR 2 WEEKS WITH ANTERIOR PITUITARY EXTRACT (PHYONE).

		Normals	Normals with 14 Injections of Phyone	Hypophysectomized, 2 Weeks	Hypophysectomized with 14 Injections of Phyone
Body weight (gm.)	Av. Range No.	2.18 1.3–3.05 15	2.45 1.18–3.4 15	2.25 1.7–3.1 14	2.21 1.3–3.3 15
Pancreas weight (mg.)	Av. Range No.	3.71±0.17 2.2–5.6 15	4.52±0.22 2.8–7.0 15	3.36±0.31 1.6–6.8 14	4.21±0.37 2.0–10.0 15
Number islets in pancreas	Av. Range No.	14.1±0.50 10–18 10	27.8±1.03 20–44 10	24.4±0.66 20–30 10	15.5±0.39 14–19 10
Number islets per mg. pancreas.		3.80	6.18	7.26	3.68
Size of islet nuclei (μ)	Av. Range No.	10.13±0.18 9.03–12.35 15	8.37±0.18 7.4–10.88 14	7.60±0.17 6.18–9.48 14	9.42±0.21 7.58–12.05 15
Ratio of number of sect. of islet tissue to total number of sect. of pancreas	Av. No.	1:2.71 10	1:1.61 10	1:1.63 10	1:2.15 10
Liver weight (gm.)	Av. Range No.	0.193±0.034 0.05–0.80 15	0.160±0.013 0.10–0.35 15	0.343±0.017 0.20–0.50 14	0.167±0.012 0.05–0.30 15
Liver glycogen weight (mg.)	Av.* No.	8.21 15	5.73 15	4.93 15	6.21 15

* Average weight of liver glycogen per individual was determined by weighing all the glycogen from each group of animals and dividing by number of animals in each group (15).

Certain histological changes also occurred. In the normal pancreas fixed according to Bowie's technique, the bulk of the island was composed of cells containing minute blue granules, not scattered throughout the cytoplasm but adhering to the large, oval, deeply-staining nucleus or to the inner surface of the cell membrane. A second type was characterized by a spotted nucleus and deep pink cytoplasm with no discrete granules. The pink cells were fewer in number than the blue ones but sometimes a small island was composed entirely of the pink type. A third type of cell had either a very pale blue or pale pink

structureless cytoplasm. According to their distribution the pink and blue cells may be likened respectively to Alpha and Beta cells (20). In both hyperpituitary and hypopituitary animals the pink type of cells seemed to be more abundant. In addition, in phyone-injected newts, there were many vacuoles in the cytoplasm of both pink and blue cells but their nuclei were not pycnotic.

In hypophysectomized animals injected with phyone, both the numerical data on the pancreas and the histological picture resembled those in normal untreated newts.

Liver. That changes in liver weight and in liver glycogen took place in the experimental animals is evident from the data of table 1. Liver weight decreased both in normal and hypophysectomized animals injected with phyone, but the decreases were not significant statistically. In the hypophysectomized group, the liver weight was significantly increased over the normal. In all of the experimental groups, liver glycogen, judged by weight and by sections, was less than in normals but in the hypophysectomized ones there was a marked increase in fat content of the cells. This may be partially responsible for the increased liver weight found in this group.

DISCUSSION

The present study reveals that the reactions of pancreas and liver to hypopituitary and hyperpituitary states of 2 weeks' duration are similar except in weights and hepatic fat content (table 2). Though the decrease in pancreas

TABLE 2. RESULTS OF HYPOPHYSECTOMY AND PHYONE-INJECTIONS OF 2 WEEKS' DURATION ON THE PANCREAS AND LIVER OF NEWTS.

	Hypopituitary State	Hyperpituitary State
Pancreas weight	Decrease	Increase
Number of islands	Increase	Increase
Size of island nuclei	Decrease	Decrease
Island cell types	More 'pink' cells	More 'pink' cells
Liver weight	Increase	Slight decrease
Liver glycogen	Decrease	Decrease
Liver fat	Increase	Normal

weight after hypophysectomy was slight and the weight increase in phyone-injected normals was barely significant statistically, both are in line with the marked decrease and increase, respectively, in size of visceral organs that characteristically occur after long periods of hypophysectomy or anterior pituitary administration (6, 7, 15). The liver response was not typical. However, its increased weight (significant statistically) two weeks after hypophysectomy seems somewhat accounted for by an increased fat content, a reaction that may possibly be attributed to lack of the ketogenic hormone of the anterior pituitary (21) or even to an injury of the hypothalamus (13). Its decreased weight after phyone injections was not significant statistically and it may be that the liver had not had time to begin its usual response in the brief injection period. In similar treatment lasting nearly the same time (12 to 20 days) no significant changes occurred in liver weights of guinea pigs (22).

All the other results (table 2) suggest an abnormal secretory activity

of islet tissue, but one probably instigated by different causes in the hypopituitary and hyperpituitary states. The islands might increase after hypophysectomy because an antagonistic action of anterior pituitary hormones toward insulin is no longer at work. The fall of blood sugar, increased sensitivity to insulin, increased tolerance to glucose (21, 23, 24) and the ability of blood of an hypophysectomized animal to lower the blood sugar of a normal one (25) point to an excess of insulin in hypophysectomized animals. Also hypoglycemia might bring about increased adrenalin secretion (26, 27, 28, 29, 30) which would cause a loss of liver glycogen (21, 31, 32) or this loss might be tied up with the adreno-cortical atrophy that occurs after hypophysectomy (13, 14, 33, 34).

If the above suggestion be correct and the same mechanism at work in the hyperpituitary state, it would seem that insulin secretion should be inhibited and that island atrophy should occur. But the anterior pituitary has some blood sugar raising capacity of its own, probably via liver glycogenolysis, for it can increase the hyperglycemia already present in pancreatectomized animals and can protect against insulin hypoglycemia (21, 24, 35). Such hyperglycemic capacity would act to induce insulin secretion (27, 36, 37, 38), eventually lower the blood sugar, and would result therefore not in atrophy but in increased numbers and new formation of islands, a response to hyperglycemia already found by Corpaci (39). Or, the islet tissue may be stimulated to increase in amount with resulting greater insulin production and therefore an eventual lowering of blood sugar, directly through a specific diabetogenic hormone, ineffective on blood sugar in the absence of the pancreas, or indirectly through a thyreotropic hormone by which thyroxin production is first increased and then island hypertrophy with greater insulin secretion follows, with concomitant blood sugar lowering, a result not secured in thyroidectomized animals (40, 41). In supporting a pituitary-thyroid-pancreas route these authors would be at variance with others (2, 26, 42) who claim that the thyroid is not involved, but would appear to agree with Aron (4) who found that the premature appearance of islets could be caused by anterior pituitary or thyroxin injections into foetal guinea pigs in utero. But whatever the method of action, the findings of the present paper on newts, those of Anselmino and his coworkers on rats (2), those of Aron (4) on foetal guinea pigs, and certain cases of hyperpituitarism described by Cecil (10), Cushing (9) and Cushing and Davidoff (11) indicate that an excess of anterior pituitary can induce increased numbers and a new formation of islets.

Furthermore the decrease in island nuclear size which assumes significant proportions in both hypo- and hyperpituitarism in newts may be an indication of overstimulation of the islets. In dogs, Allen (43), Homans (44) and Martin (45) describe an hydropic degeneration in the Beta cells of islands of pancreatic remnants incapable of preventing diabetes. The cells appear swollen, more sharply defined, less granular, then vacuolated, and later the nuclei shrink, the cell bodies break down and finally the cells disappear. Homans believed these changes were the result of exhaustion due to overstrain—an hypothesis doubted by Macleod (29)—and a somewhat similar response of

island cells of the rabbit to hyperglycemia has been described by Corpaci (39). In the present study the islands did not appear abnormal, nor were the nuclei pycnotic, although some vacuolation of cytoplasm occurred. However, more 'pink' (Alpha) cells were present, a possible indication of a relative decrease of 'blue' (Beta) cells. This fact together with the decreased nuclear size and increased number of islands seems to fit the theory of an overworked gland.

In conclusion it should be emphasized again that two different forces are probably at work in hypopituitarism and hyperpituitarism, but that the two courses of action set up by these different forces in the pancreas closely parallel each other. Hypophysectomy removes the antagonism of the anterior pituitary to insulin and, in the absence of the 'hypophyseal brake,' more insulin is liberated than normally with a resulting increase in islet tissue. In this case the blood sugar must keep pace with insulin, hence the decrease in liver glycogen. Injections of phyone, either by raising the blood sugar via liver glycogenolysis and thus creating a demand for insulin or by activating the thyroid-pancreas link or stimulating the islands themselves, cause the island tissue to overwork as shown by an increase in island number and decrease in island nuclear size. In either case liver glycogen decreases.

SUMMARY

A study of the pancreas (weight, number of islets and cellular condition) and the liver (weight, fat and glycogen content of cells) was made in 4 groups of adult male newts: normals; phyone-injected normals; hypophysectomized; phyone-injected hypophysectomized.

Compared with normals, the group hypophysectomized for 2 weeks showed a decrease in pancreatic weight, an increase in island number, a decrease in size of island nuclei, an increase in 'pink' island cells, an increase in liver weight, a decrease in hepatic glycogen and an increase in hepatic fat content. The increase in island number and in liver weight and the decrease in size of island nuclei were significant statistically.

Phyone injections into normals for 2 weeks caused an increase in pancreatic weight, an increase in island number, a decrease in size of island nuclei, an increase in 'pink' island cells, a slight decrease in liver weight, a decrease in hepatic glycogen and little or no change in hepatic fat. The increase in island number and decrease in size of island nuclei were significant statistically.

Injections of phyone into hypophysectomized animals maintained the conditions of pancreas and liver at approximately normal levels.

REFERENCES

1. Anselmino, K. J., J. Herold and F. Hoffman: Klin. Wchnschr. **12:** 1245. 1933.
2. Anselmino, K. J. and F. Hoffman: Klin. Wchnschr. **12:** 1435. 1933.
3. Hoffmann, F. and K. J. Anselmino: Klin. Wchnschr. **12:** 1436. 1933.
4. Aron, M.: Compt. rend. Soc. de biol. **113:** 1071. 1933.
5. Kojima, M.: J. Physiol. **50:** xlv. 1916.
6. Putnam, T. J., H. M. Teel and E. B. Benedict: Arch. Surg. **18:** 1708. 1929.
7. Evans, H. M., M. Simpson, K. Meyer and F. Reichert: Univ. Calif. Mem. **11:** 425. 1935.
8. Lucke, H.: Ergebn. d. inn. Med. u. Kinderh. **46:** 94. 1934.
9. Cushing, H.: Bull. Johns Hopkins Hosp. **50:** 137. 1932.
10. Cecil, R. L.: J. Exper. Med. **11:** 266. 1909.

11. Cushing, H. and L. M. Davidoff: Rockefeller Instit. Mon. No. 22: 1-131. 1927.
12. Crowe, S. J., H. Cushing and J. Homans: Bull. Johns Hopkins Hosp. **21:** 127. 1910.
13. Smith, P. E.: Am. J. Anat. **45:** 205. 1930.
14. White, W. E.: Proc. Roy. Soc. London, s.B. **114:** 64. 1933.
15. Koster, S.: Pflüger's Arch. f. d. ges. Physiol. **224:** 212. 1930.
16. Houssay, B. A. and A. Biasotti: Arch. internat. de pharmacodyn. et de thérap. **38:** 250. 1930.
17. Van Dyke, H. B. and Z. Wallen-Lawrence: J. Pharmacol. Exper. Therap. **40:** 413. 1930.
18. Bowie, D. J.: Anat. Rec. **29:** 57. 1924.
19. Zieglwallner, F.: J. Roy. Micr. Soc., p. 821. 1911.
20. Lane, M. A.: Am. J. Anat. **7:** 409. 1907.
21. Collip, J. B.: J.A.M.A. **104:** 827; 916. 1935.
22. Holden, R. F.: Proc. Soc. Exper. Biol. & Med. **31:** 773. 1934.
23. Houssay, B. A. and A. Biasotti: Endocrinology **15:** 511. 1931.
24. ————: Pflüger's Arch. f. d. ges. Physiol. **227:** 239; 664. 1931.
25. Képinov, L. and M. Guillaumie: Compt. rend. Soc. de biol. **115:** 1564. 1934.
26. Cope, O. and H. P. Marks: J. Physiol. **83:** 157. 1934.
27. Cori, C. F.: Physiol. Rev. **11:** 143. 1931.
28. Goldblatt, M. W.: J. Physiol. **79:** 286. 1933.
29. Macleod, J. J. R.: Carbohydrate Metabolism and Insulin. Longmans, Green & Co., New York. 1926.
30. ————: Bull. Johns Hopkins Hosp. **54:** 79. 1934.
31. Evans, C. L., C. Tsai and F. G. Young: J. Physiol. **73:** 61; 103. 1931.
32. Hrubetz, M. C.: Proc. Soc. Exper. Biol. & Med. **32:** 218. 1934.
33. Adams, A. E. and E. M. Boyd: Anat. Rec. **57:** Suppl. 34. 1933.
34. Loeb, R. F.: J.A.M.A. **104:** 2177. 1935.
35. Houssay, B. A., A. Biasotti and C. T. Rietti: Compt. rend. Soc. de biol. **115:** 323. 1933.
36. Banting, F. G. and S. Gairns: Am. J. Physiol. **68:** 24. 1924.
37. Kosaka, T.: J. Physiol. **79:** 416. 1933.
38. London, A. S. and N. P. Kotschneff: Pflüger's Arch. f. d. ges. Physiol. **234:** 194. 1934.
39. Corpaci, A.: Sperimentale, Arch. di biol. **86:** 129. 1932. (Abst. in Endocrinology **29:** 237, 1935.)
40. Zunz, E. and J. LaBarre: 1934. Compt. rend. Soc. de biol. **117:** 262. 1934.
41. ————: Compt. rend. Soc. de biol. **118:** 794. 1935.
42. Yriart, M.: Compt. rend. Soc. de biol. **105:** 128. 1930.
43. Allen, F. M.: J. Metab. Research **1:** 5. 1922.
44. Homans, J.: Proc. Roy. Soc. London, s.B. **86:** 73. 1913.
45. Martin, W. B.: J. Metab. Research **1:** 43. 1922.

QUANTITATIVE STUDIES ON THE STRUCTURAL CHANGES INDUCED IN THE ANTERIOR HYPOPHYSIS BY INJECTIONS OF OESTRIN[1]

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Numerous investigators (1-12) have reported that injection of oestrin or implantation of ovarian tissue into castrated female rats either prevented or caused a regression of castration changes in the anterior pituitary and that injection of sufficient amounts of oestrin induced changes in the anterior lobe comparable to those of pregnancy. Concerning the more specific action, Wolfe (13) reported that injections of large amounts of oestrin for 12 days resulted in a marked but variable weight increase in the pituitary, a finding also noted by others (10, 14, 15). Morphologically (16), it was found that the basophiles were markedly degranulated and that the relative percentages of these cells were extremely reduced. Many eosinophiles also presented loss of granules and a variable reduction in relative percentage. Many eosinophiles were partially degranulated and swollen and many had an enlarged negative image of the Golgi apparatus. The chromophobes were markedly increased both in relative percentage and in the mean number of cells counted per section. Many of these cells were enlarged and had an hypertrophied negative image of the Golgi apparatus. Large numbers of mitoses were found in the eosinophiles and the chromophobes.

We have described the action of large and small daily injections of oestrin on the anterior pituitaries of immature female rats and have pointed out that the response obtained in the anterior lobe by the injection of oestrin was at least partially dependent on the amounts injected and the duration of the injection period (17). In this paper we wish to present quantitative data on the anterior pituitaries of 3 groups of female rats; the first and second series received daily injections of large and small amounts of oestrin[2] for periods of 5 and 10 days, respectively. Members of the third group received single injections of oestrin; they were killed at daily intervals following this treatment. The general purpose of these experiments was to make a more minute examination of the comparative reaction of the various cell types of the anterior lobe to various amounts of oestrin and to determine what rôle the length of the experimental period played in the reaction obtained in the gland.

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[2] The benzoic-ester of dihydroestrin was used. A portion of this material, Progynon-B, was furnished by the Schering Corporation, Bloomfield, N.J.

MATERIALS AND METHODS

In the three series described below, complete litters of female rats 21 or 22 days old were used. At autopsy, body, ovary and pituitary weights were obtained. Serial sections of both ovaries and representative sections of the uteri and vaginæ were cut and stained. The pituitaries were prepared for study by methods previously described. Serial sections were cut and differential cell counts were made on all sections studied. From the resulting quantitative data, the relative percentages of the various cell types were calculated and were considered statistically. The sections were examined for mitoses which were counted; these data are presented as the mean number of mitoses counted per section. In addition to determining the relative percentages of the various cells, we have also attempted to obtain some idea of the actual numbers of the various cells present. Uniform methods were used in making all cell counts; every fifth field of 5 sections taken from representative areas of the gland was counted. Since this uniform procedure was used, we have obtained an index of the number of the various cells in the gland simply by calculating the mean number of each cell type counted per section. Throughout this paper the term 'mean number of cells counted per section' is considered as an index of the total number of any particular cell type present. The accuracy of this procedure depends on the technical perfection of the section and, since it is impossible to make all sections absolutely uniform or perfect, a certain amount of experimental error would be introduced. We feel, however, that in only rare instances was this error great enough to invalidate accurate conclusions.

EXPERIMENTAL RESULTS

Effects of daily small and large injections of oestrin for 5 days. Nine immature female rats received daily injections of 10 R.U. of oestrin for 5 days. The pituitaries were definitely increased in weight; the mean weight was 3.5 mg., while that in 11 controls was 2.6 mg. (table 1). Morphologically, the anterior pituitaries of the experimental rats exhibited a degranulation of many of the basophiles. The result was a decrease in the relative level of the granular basophiles to a mean of 2.4 per cent (the mean level in the controls was 8.3 per cent). Inversely, the relative level of the non-granular basophiles was increased in the experimental rats. Further reference to table 1 will show that the mean number of granular basophiles counted per section in the experimental rats was greatly reduced. The morphologic changes in the basophiles accompanying loss of granules are best understood by referring to figures 7, 8, 9 and 10 in plate 1.

Changes in the eosinophiles in the injected rats were less obvious and more difficult of analysis. A considerable number of eosinophiles were found which were swollen and partially degranulated. Such cells took a lighter stain and often presented an enlarged negative image of the Golgi apparatus (compare figures 1 and 2 with 3, 4 and 5, plate 1). Reference to table 1 will show, however, that neither the relative percentage nor the mean number of these cells counted per section was reduced; in fact, the mean levels and the number of cells counted per section were slightly increased. A possible explanation

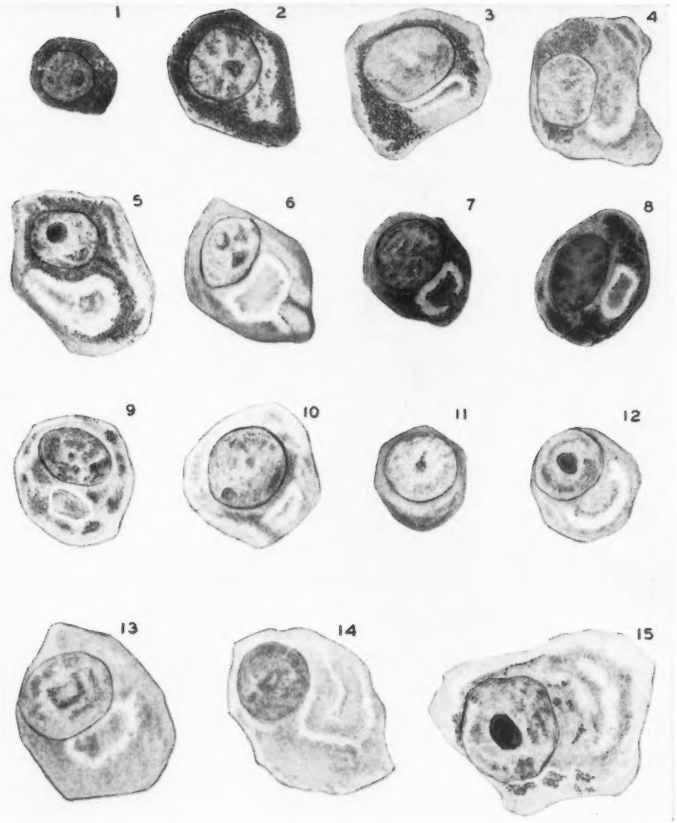

PLATE 1[3]

Fig. 1. A small eosinophile well filled with granules which are closely packed. 2. A larger eosinophile well filled with granules. The negative of the Golgi apparatus is seen close to the nucleus. 3. A large swollen eosinophile in which there is a rather marked loss of granules. The negative image of the Golgi apparatus is seen. 4. A large swollen eosinophile in which the loss of granules is marked. A portion of the enlarged negative image of the Golgi apparatus is shown. 5. Another large eosinophile showing marked loss of granules. 6. A chromophobe showing small yellowish masses in the region of the enlarged negative image of the Golgi apparatus. Near the periphery of the cell the cytoplasm was a dense blue. 7. A granular basophile. The negative image of the Golgi apparatus is visible. 8. A basophile showing a small amount of granular loss and the negative image of the Golgi apparatus. 9. A basophile showing almost complete loss of granules. The negative image of the Golgi apparatus is shown. 10. A non-granular basophile. 11. A small chromophobe with dense blue cytoplasm. The negative image of the Golgi apparatus is not clearly indicated. 12. A larger chromophobe with light blue cytoplasm and an enlarged negative image of the Golgi apparatus. 13. A greatly enlarged chromophobe with a rather dense blue cytoplasm. 14. A large chromophobe with light blue cytoplasm. The negative image of the Golgi apparatus is increased. 15. An extremely enlarged eosinophile, showing a few scattered granules and an enlarged negative image of the Golgi apparatus. All figures X 814.

[3] These cells were drawn by Miss Susan Wilkes of the Department of Illustration, Vanderbilt University School of Medicine.

of this finding was the increased rate of mitotic division in these cells in the injected rats. The mean number of mitoses counted per section in the eosinophiles of the injected rats was 14.7, while in the controls it was only 3.2 (table 1). Our findings suggest that oestrin injections tend to increase the number of the eosinophiles by increasing the rate of mitotic division and at the same time caused a decrease in the levels of these cells by inducing loss of granular material; the depleted eosinophiles giving rise to chromophobes. On the other hand, we have never found mitoses in the basophiles and in these cells we have always found that any factor which causes degranulation of these cells induces a progressive decrease in relative percentage and the mean number of cells counted per section.

The relative percentage of the chromophobes was practically the same as in the controls; the mean number of these cells counted per section was in-

TABLE 1. SUMMARY OF QUANTITATIVE DATA ON RATS RECEIVING DAILY INJECTIONS OF 10 AND 200 R.U. OF OESTRIN. THE MEAN PERCENTAGES OF CELL TYPES (M), STANDARD DEVIATIONS AND MEAN NUMBER OF CELLS COUNTED PER SECTION (C) ARE INDICATED

Injection Period	No. of Rats and Dosage	Pituitary Weight Mg.	Percentage of Cell Types				Mitoses	
			Eosin.	G. Bas.	N.G. Bas.	Chrom.	Eosin.	Chrom.
Days								
5	11 Control	2.6	M 35.2±2.2 C 287	8.3±1.3 67	1.9±0.9 18	54.6±3.3 445	3.2	7.7
	9 10 R.U.	3.5	M 37.2±2.8 C 348	2.4±1.4 25	4.8±0.8 45	55.6±4.8 510	14.7	18.4
	8 200 R.U.	3.9	M 37.1±3.0 C 314	1.0 8	5.8±1.4 47	56.1±4.1 476	11.5	13.7
10	19 Control	3.3	M 38.7±3.7 C 326	6.8±1.1 57	1.9±1.0 16	52.6±3.8 442	4.7	7.4
	17 10 R.U.	4.8	M 36.0±3.3 C 329	1.0 9	5.0±1.5 46	58.0±5.0 530	6.0	9.6
	35 200 R.U.	6.0	M 28.4±4.1 C 267	1.0 0.4	3.6±1.4 34	67.0±3.1 631	7.5	19.5

creased (table 1). The mean number of mitoses counted per section was 18.4; in the controls the mean number was 7.7. Morphologically, a considerable number of the chromophobes were swollen. Some had a fragmentary light blue cytoplasm, others a dense blue cytoplasm. The negative image of the Golgi apparatus was often enlarged (fig. 6, 11, 13 and 14, plate 1).

A second group of immature rats, littermate sisters to the control and experimental groups just described, received daily injections of 200 R.U. of oestrin daily for five days. The mean pituitary weight of this group was slightly higher than that in the group receiving 10 units (table 1). The degree of granular loss in the basophiles was greater than that in the rats receiving 10 R.U.; the relative levels of these cells in all animals were reduced to 1 per cent or below and the mean number of granular basophiles counted per section was reduced to 8 cells.

A few eosinophiles in all glands of this group exhibited loss of granules

(figs. 3, 4 and 5, plate 1), but the degree of granular loss was only slightly greater than in those rats receiving 10 R.U. of oestrin daily. The relative percentages and the mean number of these cells counted per section were slightly increased. The chromophobes were increased in the mean number counted per section but not in relative percentage. Morphologically, they were similar to those of rats receiving 10 R.U. of oestrin daily.

Effects of daily small and large injections of oestrin for 10 days. Seventeen immature rats received daily injections of 10 R.U. of oestrin for 10 days. The mean weight of the pituitaries in this group was 4.8 mg., while in the controls it was 3.3 mg. (table 1). Practically all basophiles were degranulated; in some animals no granular basophiles were found at all; in others, the relative levels of these cells were less than 1 per cent. The relative level of granular basophiles in the controls was 6.8 per cent (table 1). In the experimental rats the mean number of granular basophiles counted per section was 9, while in the controls it was 57. The non-granular basophiles were increased in relative percentage and in the mean number counted per section in the experimental rats. They appeared as large oval cells with a clear blue cytoplasm which was often fragmentary. In many the negative image of the Golgi apparatus was visible (figs. 8, 9 and 10, plate 1).

Numerous eosinophiles of the oestrin-injected rats exhibited loss of granules. However, this loss of granules was not marked enough, except in 2 rats, to lower the percentages of these cells or the mean number counted per section (table 1). The mean number of mitoses in the eosinophiles was considerably increased.

The chromophobes were increased considerably in mean number counted per section and slightly in relative percentage. The number of mitoses was also slightly increased. Morphologically, the same changes were found in the chromophobes as were found in the rats receiving oestrin for 5 days.

A second group of 10 rats, littermates to those described above, received 200 R.U. of oestrin daily for 10 days. This group was augmented by 25 other rats of identical age and treatment. The pituitaries in this series were markedly heavier than those of the controls (6.0 mg., as compared with 3.3 mg.). Degranulation of the basophiles was practically complete; the mean number of granular basophiles counted per section was only 0.4 cells. The relative percentage of the non-granular basophiles was only 3.6 per cent and the mean number of cells counted per section was only 36 (table 1). This is suggestive that in this group the basophiles lost their granules and that a majority of the resulting non-granular basophiles had passed into chromophobes.

Many of the eosinophiles also exhibited loss of granules. In most rats these cells were reduced in percentage; the mean level for the group was 28.4 per cent. The mean relative level in the controls was 38.7 per cent. The mean number of eosinophiles counted per section was also reduced (table 1). This fact was considered of significance because it revealed that relatively large amounts of oestrin administered over a fairly long period of time was necessary to reduce either the relative percentages or the mean number of these counted per section. Morphologically, many of the eosinophiles ex-

hibited various degrees of granular loss. Many showed a hypertrophy of the negative image of the Golgi apparatus and the granules took a pale stain with orange G (figs. 1, 2, 3, 4 and 5, plate 1).

Changes in the chromophobes in this group were striking. They were markedly increased in relative percentage and the mean number of cells

TABLE 2. SUMMARY OF QUANTITATIVE DATA ON RATS RECEIVING SINGLE INJECTIONS OF 10 AND 200 R.U. OF OESTRIN. THE MEAN PERCENTAGES OF CELL TYPES (M), STANDARD DEVIATIONS AND MEAN NUMBER OF CELLS COUNTED PER SECTION (C) ARE INDICATED.

Exp. Period	No. of Rats and Dosage	Pituitary Wt. Mg.	Percentage of Cell Types				Mitoses	
			Eosin.	G. Bas.	N.G. Bas.	Chrom.	Eosin.	Chrom.
Days								
	5 Control	2.6	M 37.6±3.6 C 268	8.2±0.9 58	1.8±1.5 13	52.4±3.3 374	1	7
1	3 10 R.U.	2.6	M 39.2±4.7 C 295	5.7±0.9 43	5.0±1.6 38	50.1±2.1 377	1	4
	6 200 R.U.	2.9	M 38.4±4.5 C 302	4.3±2.5 34	6.0±3.0 47	51.3±4.4 406	2	20
	5 Control	2.5	M 34.5±2.4 C 261	6.3±2.0 48	2.6±1.9 20	56.6±2.8 428	3	8
2	3 10 R.U.	2.8	M 35.9±4.7 C 284	2.7±1.5 22	5.2±1.6 42	56.2±4.8 456	6	12
	2 200 R.U.	3.3	M 35.0±2.5 C 287	3.0±2.0 35	7.0±1.6 57	55.0±2.5 451	12	19
	3 Control	2.5	M 39.6 C 269	10.6 72	0.4 3	49.4 336	0	3
3	3 10 R.U.	2.5	M 40.0±2.5 C 308	4.0±1.0 31	5.0 38	51.0±2.5 393	8	6
	4 200 R.U.	3.1	M 40.5 C 297	3.5±1.6 26	6.0±1.0 44	50.0±1.7 367	18	16
	3 Control	3.0	M 34.2±2.5 C 283	7.0±1.6 58	1.7±0.9 14	57.1 473	1	5
4	3 10 R.U.	3.5	M 37.0±2.5 C 334	7.0 63	4.0±1.0 36	52.0±2.5 469	7	5
	4 200 R.U.	4.0	M 38.8±7.0 C 359	1.0 9	6.5±0.8 60	53.7±5.4 497	16	14
	3 Control	2.4	M 35.9±2.3 C 298	7.6±0.9 63	2.3±0.9 19	54.2±5.7 451	4	8
5	5 10 R.U.	2.6	M 40.5 C 341	4.6±3.0 39	6.4±1.4 54	48.5±5.1 409	11	5
	4 200 R.U.	3.5	M 42.0 C 377	2.5±1.6 22	5.5±1.6 49	50.0±43. 448	17	12

counted per section. Many cells were markedly enlarged. In some the cytoplasm was fragmentary and almost colorless; in others it was more dense and took a deeper stain. In some cells small clumps of yellowish material was found in the region of the Golgi apparatus. In both the large and small chromophobes the negative image of the Golgi apparatus was often enlarged (figs. 6, 11, 12, 13 and 14, plate 1).

Effects of a single small and large injection of oestrin on animals killed within 5 days after injection. The experiments described above revealed that

daily injections of either 10 or 200 R.U. of oestrin for periods of 5 or 10 days caused loss of granules from the basophiles and the eosinophiles. However, it was much easier to induce degranulation of the former than of the latter cells; i.e., 10 R.U. daily for 5 days induced a very definite loss of granules from the basophiles, while changes in the eosinophiles were relatively slight. Therefore, it seemed possible by employing still smaller amounts of oestrin it might be possible to induce changes in the basophiles without bringing about coincident changes in the eosinophiles.

A series of 14 immature rats received a single injection of 10 R.U. of oestrin; various members of this group were killed on days 1, 2, 3, 4 and 5 following injection. Nineteen littermate controls were killed at the same post-injection intervals. Reference to table 2 will show that a single injection of 10 R.U. of oestrin induced a relatively slight weight increase of the pituitary which was evident in rats killed on days 2, 3, 4 and 5 following injection. A slight loss of granules from the basophiles and a reduction in the relative level of the basophiles was discernible in rats killed 24 hours after injection; both the morphologic and the quantitative findings were much more evident in rats killed 2 days and later following injection. Coincident with the decrease in the relative percentages and the mean number of cells counted per section in the granular basophiles, there were increases in the non-granular basophiles. In this group of rats there was no morphologic evidence of loss of granules from the eosinophiles. In none of the injected rats was there any evidence of reduction of the relative level of the eosinophiles; in fact, the mean number of these cells counted per section was slightly increased (table 2). Correlated with this fact was an increased rate of mitotic division in the eosinophiles. Changes in the chromophobes were relatively slight. There were a few swollen cells and a very few exhibited a hypertrophied negative image of the Golgi apparatus. The rate of mitotic division in these cells was increased (table 2).

Another group of 19 rats, littermate sisters to those described above, received a single injection of 200 R.U. of oestrin. They were injected and sacrificed at the same time as the above experimental and control animals. Table 2 will show that the pituitary hypertrophy resulting from the single injection of 200 R.U. was considerably greater than that in rats receiving the single injection of 10 R.U. of oestrin. Morphologically, the degree of degranulation of the basophiles and the reduction in the relative percentage and the mean number of granular basophiles counted per section was consistently and definitely greater than in the rats which received 10 R.U. of oestrin (table 2). Inversely, there was a rise in the relative level of the non-granular basophiles in the injected rats. Analysis of changes in the eosinophiles shows that the relative percentages of the cells were not reduced in the rats receiving 200 R.U. of oestrin; the mean number of these cells counted per section was slightly greater than in the control rats. Morphologically, the eosinophiles showed no evidence of loss of granules in the rats killed on days 1, 2 and 3 following injection. However, a majority of those killed on days 4 and 5 following injection showed loss of granules from relatively few eosinophiles. Changes in the chromophobes were slight; a few

cells were swollen and a considerable number presented a negative image of the Golgi apparatus which was definitely increased in size. The rate of mitotic division was increased in both the eosinophiles and the chromophobes of the oestrin-injected rats (table 2).

SUMMARY

Two groups (total of 17) of immature female rats received daily injections of 10 and 200 R.U. of oestrin, respectively, for 5 days; 11 littermates served as controls. In rats receiving 10 R.U. daily there was a moderate basophilic degranulation and loss of granules from relatively few eosinophiles. In rats receiving 200 R.U. the degranulation of both the basophiles and the eosinophiles was more evident.

In a second series, 2 groups (total of 52) of rats received daily injections of 10 and 200 R.U. of oestrin, respectively, for 10 days; 19 littermates served as controls. In rats receiving 10 R.U. daily practically all the basophiles and moderate numbers of the eosinophiles were degranulated. In rats receiving 200 R.U. the basophilic degranulation was complete and many of the eosinophiles showed loss of granules.

In a third series, 2 groups (total of 39) received a single injection of 10 and 200 R.U. of oestrin, respectively. Various members of these 2 groups were killed on days 1, 2, 3, 4 and 5 following injection. Nineteen littermate controls were used. Single injections of 10 R.U. induced degranlation in a moderate number of the basophiles; changes in the eosinophiles were not noteworthy. Single injections of 200 R.U. induced a more extensive degranulation of the basophiles and loss of granules from a few eosinophiles in the rats killed on days 4 and 5 following injection.

Differential cell counts were made on all sections studied. The relative percentages and the mean numbers of the various cell types counted per section were calculated. Quantitative data were presented statistically. The pituitaries of the oestrin-injected rats were increased in weight when compared with those of littermate controls. The degree of this weight increase was proportional to the amounts of oestrin injection and the length of the experimental period.

REFERENCES

1. Lehman, J.: Pfluger's Arch. f. d. ges. Physiol. **216:** 729. 1927.
2. Baniecki, H.: Arch. f. Gynäk. **134:** 692. 1928.
3. Baniecki, H.: Arch. f. Gynäk. **129:** 478. 1932.
4. Hohlweg, W., and Dohrn, M.: Wein. Arch. f. inn. Med. **21:** 337. 1931.
5. Haterius, H. O., and Nelson, W. O.: Anat. Rec. **61:** 175. 1932.
6. Nelson, W. O.: Anat. Rec. **35:** 31 (Suppl.). 1933.
7. Nelson, W. O.: Proc. Soc. Exper. Biol. & Med. **32:** 452. 1934.
8. Friedl, F.: Ztschr. f. Geburtsh. u. Gynäk. **105:** 227. 1933.
9. Bachner, F.: Ztschr. f. Geburtsh. u. Gynäk. **106:** 87. 1933.
10. Halpern, S. R., and F. E. D'Amour: Proc. Soc. Exper. Biol. & Med. **32:** 108. 1934.
11. Halpern, S. R.: Anat. Rec. **61:** 21. (Suppl.) 1935.
12. Wolfe, J. M.: Proc. Soc. Exper. Biol. & Med. **32:** 1189. 1935.
13. Wolfe, J. M.: Proc. Soc. Exper. Biol. & Med. **32:** 757. 1935.
14. Hohlweg, W.: Klin. Wchnschr. **13:** 92. 1934.
15. Selye, H., J. B. Collip and D. L. Thomson: Proc. Soc. Exper. Biol. & Med. **32:** 1377. 1935.
16. Wolfe, J. M.: Proc. Soc. Exper. Biol. & Med. **32:** 1192. 1935.
17. Wolfe, J. M.: Proc. Soc. Exper. Biol. & Med. **34:** 56. 1936.

THE RELATIONSHIP OF THE ANTERIOR PITUITARY GLAND TO THYROID AND OVARY[1]

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Extracts of anterior pituitary of cattle were shown by Evans to produce corpora lutea or corpus luteum-like structures in the ovary, and by Ascheim and Zondek, as well as by Smith, to cause the growth and maturation of follicles. It was the rat and mouse which served as the test animals in these investigations. In the guinea pig, on the other hand, it could be shown that injections of similar extracts caused in a short time a remarkable transformation of the thyroid gland into one resembling that found in extreme cases of Graves' disease, a change which could not be obtained in rat, mouse or rabbit and which made much more feasible an experimental study of the stimulating effect of the anterior pituitary gland on the thyroid.

It is the intention here to describe a series of investigations which were carried out mainly with the guinea pig instead of with rat, mouse and rabbit and in which a simple method was used that makes easily possible the study of certain problems which would be difficult to approach by means of the commonly employed methods. This method consists in the immersion of individual anterior pituitary glands, irrespective of their size, in various solutions and in the subsequent implantation of these glands into the test animal, primarily the guinea pig. It can be shown that as the result of this preliminary treatment the anterior pituitary gland has undergone definite changes which can be demonstrated through their effect on thyroid and ovary after implantation. The anterior pituitary of small laboratory animals can be analyzed by means of this method as well as single glands of larger animals, such as cattle, sheep, and hog. We then wish to analyze largely by means of this method the relations between ovarian hormones and the thyroid-stimulating hormone of the anterior pituitary gland and to study in particular a substance present in certain anterior pituitary glands which instead of causing growth and maturation of follicles induces their destruction in the ovary of the guinea pig.

The guinea pig differs from mouse, rat and rabbit in that its sexual cycle is more complete and typical than that of the latter animals and that its ovary is in certain respects a more sensitive and responsive test organ than the ovary of rat and mouse. When we inject either acid or alkali extracts, neutralized subsequently, of anterior pituitary gland from cattle, sheep or pig, intraperitoneally into guinea

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pigs there occurs, instead of a growth and maturation of the follicles, an almost complete atresia; only the very small follicles remain preserved and may again grow to full size later. Such a picture in the ovary of the guinea pig is not unlike that seen at the time of ovulation, in which a fargoing destruction of follicles also occurs caused, presumably, by circulatory difficulties. Likewise following a very energetic process of underfeeding leading to a great loss in weight in young guinea pigs, gradually a hypotypical condition of the ovary may result. However after injection of anterior pituitary extract from cattle, sheep or hog, we find, in addition to the fargoing atresia of follicles, other changes, namely, a rudimentary development of interstitial gland in the medulla, some rings of lutein tissue at the inside of the theca interna in follicles in the stage of connective tissue atresia, and structures transitional between such follicles and small pseudo-corpora lutea, as well as occasional medium sized pseudo-corpora lutea. Exposure of the guinea pig ovaries to Roentgen rays may produce similar changes. Implantation of the anterior pituitary of cattle as such, instead of injection of extracts gives similar results, although they may be slightly mitigated. In the anterior pituitaries of cattle, sheep and pig a substance is therefore present which leads to an injury of ovarian follicles and causes a fargoing atresia; associated with these changes are some slight luteinization processes. These extracts induce at the time time a marked hypertrophy and hyperplasia of the thyroid gland and a solution and absorption of its colloid.

If, instead of injecting the anterior pituitary extracts from cattle, sheep or pig, we implant anterior pituitary of rat or rabbit, we obtain an entirely different picture. There develop very large, fully mature follicles. The growth and maturation of follicles may be followed by their rupture, but this seems not to be the usual effect. As a rule no rupture takes place in these follicles but, instead, capillaries and connective tissue cells grow into the mature granulosa from the theca interna and transform the whole structure first into a pseudolutein body and gradually into a solid pseudocorpus luteum. But in addition other changes take place in ovaries of guinea pigs into which normal anterior pituitaries of rat and rabbit or anterior pituitaries of cattle or man previously treated with various substances have been implanted. Theca interna cells in various places, remnants from atretic follicles which had been pushed into the medulla, may hypertrophy and multiply thus producing interstitial gland tissue. The theca interna around follicles in the process of atresia and sometimes even around growing follicles may undergo hyperplastic and hypertrophic changes. At the same time the granulosa of the latter follicles may enlarge somewhat and become mature and then luteinize prematurely in smaller follicles or also in larger ones. This hypertrophy in the granulosa is often less marked than in the surrounding theca interna; in this case granulosa and theca interna are distinct from each other and when connective tissue and capillaries grow into the granulosa, as so often occurs, a process develops which can be compared with the connective tissue growth taking place in ordinary follicles during the process of atresia. We may designate the former process as luteinizing connective tissue atresia, because in both cases the end result is the destruction of the follicles under the influence of the ingrowing connective tissue. However, if the luteinization process in the granulosa becomes as pronounced as that in the theca interna and a subsequent ingrowth of vessels and connective tissue occurs, pseudo-corpora lutea of various sizes are produced, in which the luteinized theca interna and granulosa can no longer be distinguished from each other. In the luteinizing connective tissue atretic follicles the central connective tissue gradually shrinks; in addition a part of the granulosa becomes apparently replaced by connective tissue and in the end

there remain interstitial-gland bodies consisting largely of luteinized theca interna, but in which there may be added remaining parts of luteinized granulosa. Interstitial-gland bodies of a similar appearance may perhaps owe their origin to a secondary luteinization of the theca interna in follicles in the later stages of connective tissue atresia, in which therefore the granulosa does not participate in the production of the luteinized tissue. Commonly, as stated above, under the influence of implanted anterior pituitary glands a premature maturation of granulosa also occurs; it can be seen in follicles of small-medium and medium size instead of in large-sized follicles where it normally takes place. These premature changes in the granulosa again may be followed by ingrowth of connective tissue and capillaries and may again ultimately lead to the production of interstitial-gland bodies representing follicles in late stages of connective tissue atresia.

We may then classify the changes found in the ovary of the guinea pig into 4 kinds: a) growth and maturation of large follicles; b) production of true corpora lutea, pseudolutein bodies, and pseudocorpora lutea, representing the first type of luteinization; c) premature maturation and luteinization of granulosa, luteinizing connective tissue atresia, formation of interstitial gland bodies and of bodies transitional between the two last named structures and small pseudocorpora lutea as well as development of strands of interstitial gland in the medulla, all these formations representing the second type of luteinization; d) advanced atresia of follicles, as described above. The luteinization processes consist essentially in maturation and subsequent luteinization changes in theca interna as well as in granulosa; the first and second luteinizations differ in the size of the follicles which are affected by these changes. In the second luteinization process these changes are not limited to large follicles approaching maturity but they affect growing follicles of as yet smaller size. Furthermore, there are differences as to the relative rapidity and intensity with which theca interna and granulosa respectively luteinize.

In the anterior pituitaries of cattle, sheep and pig, substances which cause the fourth, the destructive process, predominate with a slight addition of the second type of luteinizing processes. The anterior pituitaries of rat and rabbit produce a combination of processes a, b and c (growth of follicles and both luteinization processes). Anterior pituitaries of cat and man act similarly. However after the implantation of human anterior pituitaries the pure maturation effect found in large follicles may be somewhat less prominent although it may be present, than after implantation of rabbit, rat and guinea pig anterior pituitaries. The anterior pituitary of the guinea pig contains predominantly the growth-maturation producing substances with only a slight admixture of the first and second luteinizing hormone, although ovulation may relatively readily occur after implantation of guinea pig anterior pituitaries. Only by daily implantation of many guinea pig anterior pituitaries can the second luteinization products be made prominent. Urine of pregnant women exerts principally the effects of second luteinization, which are, however, not limited to changes in the theca interna, but in which also the granulosa may play a certain part; a full maturation of large follicles does not as a rule occur. We notice furthermore a certain destructive action exerted on the granulosa following the first two injections of this substance in the guinea pig.

There is a definite relation between the action of these anterior pituitary glands on the ovary and on the thyroid gland. The strongest action on the latter organ is exerted by the extracts of cattle, sheep and hog anterior pituitaries. Urine of pregnancy is entirely devoid of this action and it is very slight in the case of the guinea pig anterior pituitary. The others are intermediate. The effect on the thyroid is usually

marked after implantation of human anterior pituitary but this may be partly due to the relatively large size of this organ as compared with the anterior pituitaries of rabbits or rats. If we compare the effects of equal amounts of cattle and human anterior pituitary, it is found that those exerted by cattle anterior pituitary as a rule are greater. Those anterior pituitary glands which contain predominantly the second luteinizing and the follicle-destroying substance cause the most pronounced hypertrophy of the thyroid gland, while those which contain predominantly follicular growth-maturation substance, such as the guinea pig anterior pituitary, have the least effect on the thyroid gland. This association between the thyroid-stimulating and the second luteinizing hormone and the follicle-destroying substance may be observed in many other cases and especially also under experimental conditions.

Anterior pituitary glands of different species differ, then, considerably in regard to the preponderance of the substances causing the various effects in ovary and thyroid. It might be expected that this difference in the constitution of the anterior pituitaries should affect also the normal structure and function of the recipient organs and there is indeed an indication that this is the case. Thus the structure of the thyroid gland in the guinea pig has more definitely the characteristics of a resting gland than the thyroid gland in rat, mouse or rabbit in accordance with the lesser amount of thyroid stimulating hormone present in the guinea pig anterior pituitary. Moreover there seems to exist, as pointed out by us several years ago, an inverse relation between the activity of the anterior pituitary and the reactivity of the thyroid gland in a certain species, so that within certain limits the product of both is approximately constant. Furthermore, in the ovary of guinea pig, in the anterior pituitary glands of which the luteinizing substance is present in a much smaller quantity than in the other species, the formation of the interstitial gland is much less developed than in the rat, mouse and especially rabbit, in which luteinizing substances are present in a larger quantity in the anterior pituitaries.

If we compare with the action of the various anterior pituitary glands on the ovary of the guinea pig, their effect on the ovary of the rat, we find in principle a correspondence between the results obtained in both. But while in the guinea pig these differences are clear and to a certain extent quantitative in character, in the rat they are much less distinct and may be merely indicated.

It is possible to change the effects of the anterior pituitary glands of the various species experimentally by immersing the glands in various solutions before implanting them into the guinea pig. As stated, injection of extract or implantation of sufficiently large pieces of cattle anterior pituitary invariably causes a marked destruction (atresia) of the ovarian follicles. But if we immerse such a gland for 1 to 3 days or longer in various solutions such as water, 0.9 per cent NaCl (if necessary with addition of small amounts of preservative substances), alcohol, ether or certain other substances or combinations of substances we obtain a different result. Instead of an almost pure follicular atresia we now observe a predominance of the various luteinization effects and, in certain cases, we may observe even production of large, well preserved follicles. Still different is the result obtained if we first immerse the anterior

pituitary cattle gland in 1 or 1.5 per cent solution of formalin for 3 to 7 days. After immersion in the latter solutions the thyroid-stimulating hormone is destroyed or inactivated and also the follicle destruction and second luteinization processes are abolished. There remains then mainly or exclusively the action of the follicular growth-maturation hormone, which seems to be perhaps protected or enhanced in its action. If we implant such a gland into a young immature guinea pig we may observe the production of very large mature follicles. Also immersion of the human anterior pituitary in 50 per cent alcohol saturated with Na_2SO_4 seems to favor the predominance of the growth-maturation producing substances over the second luteinizing substances. Certain differences in the results may be observed in accordance with certain variations in the anterior pituitary glands or in the guinea pigs into which the anterior pituitary is implanted. Thus a good state of health and a somewhat greater weight of the recipient animal seems to favor, in general, a good growth of follicles, while a low initial weight and a loss in weight during the experiment on the whole favors a less good development of the follicles and the follicular atresia. In the case of the formalin action a full maturation of the follicles is not obtained in every case; there may be merely a development of large, not mature follicles. In the case of a number of substances there seems to be a balance between the two antagonists, namely, the follicle-destroying and the follicular growth-maturation producing substances. It may depend upon variable external factors, whether the one or the other of these two substances predominates. But, on the whole, the principal results are clear cut and predictable under this condition of experimentation.

Human anterior pituitary behaves in principle in the same way as cattle anterior pituitary but there are some differences between the action of these two glands after they have been subjected to the same preliminary treatment. In the human anterior pituitary there is, on the whole, a somewhat greater preponderance of the growth-maturation substance than in the cattle anterior pituitary, while in the latter the thyroid-stimulating hormone and the follicle-destroying substance are more predominant. However, as stated also in the cattle anterior pituitary the follicular growth-maturation substance is present in considerable quantity, but under ordinary conditions its action does not become manifest in the guinea pig, because the follicle-destroying substance suppresses this effect. As to the thyroid-stimulating hormone, in accordance with what we stated previously, it is usually associated with the follicle-destroying and luteinizing substances. However, it is possible to dissociate it from these two substances. After elimination of either of them the thyroid-stimulating hormone may still be present; also normally it occurred in the thyroid of a puerperal subject, although the luteinizing substance was almost completely lacking. Similarly in the anterior pituitaries of certain children before the age of puberty the follicular growth-maturation substance may be present almost exclusively and at the same time some thyroid-stimulating hormone may be found. Or, in transitional cases, when the formalin action is not yet fully developed, the thyroid hormone may be destroyed while the luteinizing hormone may still be active. In general, formalin seems to injure

the thyroid-stimulating hormone earlier and more severely than the luteinizing substance. The fact that the thyroid-stimulating substance is distinct from the luteinizing hormone agrees also with the recent work of Greep who, on the basis of the studies of Hisaw and Fevold, showed that it is possible to separate two extracts of anterior pituitary one of which acts only on the thyroid gland while the other contains the luteinizing hormone. However, these conclusions rest on experiments with the rat as far as the changes in the ovaries are concerned. It would be desirable to compare with these observations the effects of such extracts on the guinea pig ovary.

After immersion in a large number of substances the action of human and of cattle anterior pituitary glands may become very similar. Formalin acts on both kinds of glands in the same direction and so do also the other substances which we mentioned as well as some additional ones, which in the case of cattle gland to a large extent cause an elimination or weakening of the follicle-destroying substance in addition to other effects and which in the case of the human gland tend to inactivate the follicular-growth substance.

It is thus possible by means of this treatment to bring out effects and characteristics of the anterior pituitary gland which remain hidden under normal conditions. It is to be expected that the follicle-destroying substance prevents the action of the follicular growth-maturation substance not only by its destructive effect on granulosa but also by inhibiting the growth of the theca interna; but there are, furthermore, indications that the luteinizing substance to a certain extent retards the follicular growth-maturation hormone in its action. This may be accomplished by the premature maturation of the smaller follicles and by an early luteinization of the theca interna, changes which are unfavorable to further growth of the follicles. It is therefore necessary to remove in succession these inhibiting substances in order to allow the growth-maturation hormone to become effective. The effects which the anterior pituitary glands of various species exert depend therefore largely on the relative mixture of these three substances, the follicular growth-promoting, the follicular growth-inhibiting and the luteinizing substances. As we have seen, the relative predominance of these substances can be modified experimentally by immersing the glands in various solutions before implanting them. However, it is also possible to vary the relationship between these three substances and cause a certain shifting process among them, by varying the quantity of of anterior pituitary substance which we implant. Thus if we diminish, by one-half, the amount of cattle gland implanted daily, the second luteinization processes in theca interna may be somewhat accentuated and the development of small interstitial gland bodies be promoted; also the interstitial gland in the medulla may thus be made somewhat more prominent. Evidently by diminishing the quantity of anterior pituitary we diminish the effects of the atresia-producing substance to a greater extent than those of the luteinizing substance and the latter become therefore more pronounced.

Which of the various substances will predominate depends, however, not only on the experimental treatment which the anterior pituitary glands undergo before implantation but also on the species character of the recipient ovary.

Similar differences are found therefore in the ovary as were found previously in the case of the thyroid gland. Thus in the rat the follicle-destroying substance is much less effective than in the guinea pig; presumably because in the former the granulosa is much more resistant to injurious conditions in general than in the guinea pig. Moreover, there is some indication that the readiness with which ovulation can be induced in the various species differs; we have the impression that ovulation may perhaps be a more common effect in mouse and rabbit than in the guinea pig, in which latter species under ordinary conditions the number of ovulations following mating is smaller than in the rabbit, rat and mouse. However, this may be taken merely as a suggestion which needs further study.

We see then that the effects which, under ordinary conditions, an anterior pituitary gland exerts on the ovary are no indication of the various active substances which may be present in it and the potentialities it possesses. This can readily be brought out experimentally by the method described. It is furthermore seen that it is possible to transform the action of the anterior pituitary gland of one species into that of another species by subjecting the gland to the influence of certain substances before implanting it. Thus the effect of cattle anterior pituitary can be changed experimentally into those of human or of guinea pig anterior pituitaries, and the effects of human anterior pituitaries can be changed into those of guinea pig anterior pituitaries and perhaps even into those of cattle anterior pituitary.

This method makes it possible in a relatively simple manner to analyze single glands from larger species and only a relatively small number of glands from small laboratory animals is needed for this purpose. It can be used therefore under conditions which would hardly allow the preparation of many different extracts. Furthermore the presence of hormones and perhaps other substances can thus be demonstrated in anterior pituitary glands which can not be readily extracted with either a dilute acid or alkali but which, nevertheless, are contained in the gland. However, the best results may be expected if both methods are used side by side.

To turn again to the follicle-destroying substance found in cattle anterior pituitary, it is able to inhibit the effect of growth-maturation substance of rat, guinea pig or rabbit anterior pituitary. This inhibiting effect can be made manifest, when we inject bovine anterior pituitary extracts, either acid or alkali, and at the same time implant the anterior pituitaries of the rodents named or even when in a first period we inject the bovine extract and in a second period implant rabbit or guinea pig anterior pituitaries. The growth-maturation effect is thus prevented, but the second luteinization processes are not prevented thereby (Helen M. Aff and Leo Loeb). In this connection we may recall some experiments of P. E. Smith (1927) and of Evans and Simpson (1928) in which they showed that an extract containing the bodily growth-promoting principle of Evans inhibited early sexual maturation ordinarily induced by implantation of rabbit or rat anterior pituitaries in immature rats. These experiments suggested to Evans that an antagonism exists between the growth hormone and the follicular growth-maturation hormone of the

anterior pituitary. However, in the light of the experiments which we have reported, we would attribute these inhibiting effects to the presence of a special follicle-injuring substance in the anterior pituitary of cattle, sheep and hog. This atresia-producing substance might provisionally be designated as 'Atresin.' Its presence can be demonstrated not only in extracts but also in the anterior pituitary glands as such. It can be shown to exist in purified cattle or sheep anterior pituitary extracts, such as those prepared by Collip and Lacqueur. While this substance can be readily obtained from the anterior pituitary glands of these species, it is not certain that in the normal organism it is given off by the hypophysis and acts as a hormone. Hayward and Loeb have not been able so far to find a substance comparable in strength to atresin in normal cattle kidney and spleen, either in the whole tissue or in extracts prepared from it. There are indications that it is possible to extract atresin from cattle anterior pituitaries in such a way that only very slight amounts of luteinizing and thyroid stimulating hormone are extracted at the same time. However, these investigations are in an early stage and these statements must therefore at present be considered as provisional.

SUMMARY

The results of an elaborate series of experiments are reported in which, among others, the following findings were made.

There exists, in addition to the growth-maturation and luteinization-producing hormones, a substance in the anterior pituitary mainly of cattle, sheep and pig, which, in the guinea pig ovary, causes a rapid destruction of follicles. There are indications that extracts may be obtained from cattle glands which contain this substance in an effective concentration, while the thyroid-stimulating and the luteinizing-substances are present only in very small amounts. This follicle-destroying substance may be designated as *'Atresin.'*

While as a rule the thyroid-stimulating hormone is found in association with the luteinizing hormone and with atresin and its occurrence shows an inverse relation to that of the follicular growth-maturation hormone, it is possible experimentally to separate these three substances. Thyroid-stimulating hormone may also in certain human anterior pituitaries (puerperal women, children before puberty) be associated almost altogether with the follicular growth-maturation hormone; the same combination was observed in some human glands that were treated with solutions of alcohol saturated with Na_2SO_4. On the other hand, it can be shown experimentally that the luteinization hormone may be active without any indication of the presence of thyroid-stimulating hormone. We may therefore conclude that all these hormones may act independently of each other .

In addition to the usual method of preparing extracts of anterior pituitary glands and thus analyzing the hormones present in these organs, a simple method has been devised which allows an analysis of the active substances contained in these glands under conditions more nearly approaching the normal state, where the hormones are elaborated in and acted upon in the

tissues. By means of this method, it is possible to analyze single glands in larger species and a smaller number of glands is sufficient for analysis in smaller species and it is possible thus to carry out a considerable number of experiments in a relatively short time. This method may be of still greater value if used in combination with the preparation of extracts.

By means of this method it has been possible to demonstrate in cattle anterior pituitary glands not only the presence of atresin, but also of the other two hormones. It can be shown that the presence of atresin may hide the action of the other hormones and the presence of the luteinizing hormone may cover up the presence of the follicular growth-maturation hormone. It is also by means of this method possible to transform the effects of one type of anterior pituitary gland into that of another type.

ASSAY OF BLOOD AND URINE FOR THYREOTROPIC HORMONE IN THYROTOXICOSIS AND MYXEDEMA[1]

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Rogowitch (1) demonstrated hypertrophy of the anterior pituitary body following thyroidectomy in rabbits and dogs as early as 1889. Nièpce (2) in 1851 recorded that both man and animals with large parenchymatous goiters had greatly enlarged pituitaries. Boyce and Beadles (3) in 1892 described enlargement of this gland along with colloid in the anterior lobes of the pituitary in cases of human myxedema. This finding was confirmed in 1917 by Hale White; and Wegelin (4) described similar findings in a case of cachexia thyreo priva. Degener (5), Kojima (6) and Kamo confirmed the findings of Rogowitch in animals.

In 1914 Adler (7) demonstrated that destruction of the pituitary in tadpoles depressed metamorphosis and the development of the thyroid gland. Allen (8) and Smith (9) in 1916 independently studied the same relationship in tadpoles. Gudernatch (10) had described the rôle of the thyroid hormone in metamorphosis in 1912 and it was therefore logically suspected that the inhibition of metamorphosis following pituitarectomy was in some way related to secondary diminution in thyroid function. Allen's work (11) in 1921 indicated that pituitary transplants induced premature metamorphosis following thyroidectomy only if some residual tissue was present. Smith and Smith (12) were able to induce metamorphosis in pituitarectomized tadpoles by the intraperitoneal administration of anterior lobe of sheep. Schwartzbach and Uhlenhuth (14) showed that thyroidectomized salamander larvae could not be made to undergo metamorphosis with large doses of pituitary extracts which were readily effective in the presence of the thyroid.

In extending the above concepts to mammals Smith (15) clearly demonstrated that following pituitarectomy in the rat regression could be prevented or repaired by implantation of anterior lobes of rat pituitaries. Aron (16) demonstrated thyreotropic activity of anterior pituitary extracts in the guinea pig. Later studies by Smith and Siebert (17) indicated that extracts of anterior lobe substance increased the metabolic rate in the presence of the thyroid in the guinea pig.

Schockaert (18) reported the production of a syndrome of exophthalmos, thyroid hyperplasia, decreased thyroid iodine and increased metabolic rate in the duck. He noted the tendency of the hyperplastic thyroid to involute in a few of his animals after prolonged treatment with anterior pituitary material. Schockaert drew the analogy between anterior pituitary induced hyperthyroidism and Graves' disease in the human.

Histologic studies by Hertz and Kranes (20) pointed to an exhaustion of the

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thyroid epithelium after prolonged administration of the thyreotropic factor. The 'natural remission' despite prolonged thyreotropic administration had been noted in the metabolic studies of Friedgood, Loeb and Collip. Friedgood (21) published metabolic data and an account of anterior pituitary-induced exophthalmos and hyperthyroidism in the guinea pig. He also drew attention to the cyclic nature of the thyroid response to stimulation by the thyreotropic factor in a later publication (22).

Attempts to extend the anterior pituitary hypothesis to the pathological mechanism operative in human Graves' disease have been made. Marine (24) in a recent review on thyroid physiology stressed the resemblance between the hyperthyroidism produced in animals by injection with thyreotropic hormone and exophthalmic goiter in man and infers that there is an over-production of thyreotropic principal in Graves' disease. He suggests as an alternative possibility that exophthalmic goiter may occur in man when the capacity to produce an antithyreotropic material is impaired. He also states that a decreased production of thyreotropic hormone is probably the immediate cause of myxedema.

Aron (25) tried to obtain evidence of increased thyreotropic activity of the pituitary in human cases of Graves' disease. He used young guinea pigs as test animals and depended upon minor histologic changes such as vacuolization of the colloid as criteria in reading the test. It was with a similar view that the studies of Krogh and Okkels (26) were made. In their early experiments they attempted to repeat the technique of Aron. They agreed that there was no evidence of increased thyreotropic material in the urine of patients with Graves' disease, but added that the histologic assay method was not reliable. By using metabolic rate determinations in their assay technique these workers could find no further evidence of thyreotropic hormone in the urine of thyrotoxic patients, even after its concentration.

Castillo and Magdalena tried to verify Aron's work and used his method of assay. Their results forced them to the conclusion that guinea pigs were unsuitable for assay of thyreotropic action of sera. They found that of 10 hypophysectomized dogs, 3 had sera which gave a positive reaction by Aron's technique and criteria. Obviously the results of Aron must be regarded in the light of this nonspecificity of his assay method.

We therefore devised our own method of assay for thyreotropic factor using the pituitarectomized rat as test object. In so doing we followed the work of Collip and his co-workers in their attempts to purify thyreotropic hormone from pituitary tissue. This method can be regarded as specific for thyreotropic factor since it depends upon a principle similar to that underlying the substitutional transplant method used by Smith in his original contributions to the thyreotropic field.

METHOD AND RESULTS OF ASSAY

The method of assay which we finally developed and consider suitable for assay of biologic fluids for thyreotropic activity is as follows. White rats of Wistar strain were used throughout our experiments. Males weighing between 150 and 200 grams were selected. They were pituitarectomized by a modified Selye* technique. Five days were allowed postoperatively for recovery. Intramuscular injections of urine or serum were then instituted and carried on

* We wish to express our thanks to Professor J. B. Collip and Dr. H. Selye working in his laboratory for their many kindnesses extended to us at the inception of this study, and to Professor J. H. Means for his review of our material and stimulating suggestions.

over a period of 5 days. On the 10th or 11th day the animals were sacrificed and their thyroid glands obtained for histological examination. In our experience 2 injections daily of 5 cc. of untreated fresh urine and of 1 or 2 cc. of blood serum were well tolerated by pituitarectomized animals in good condition.

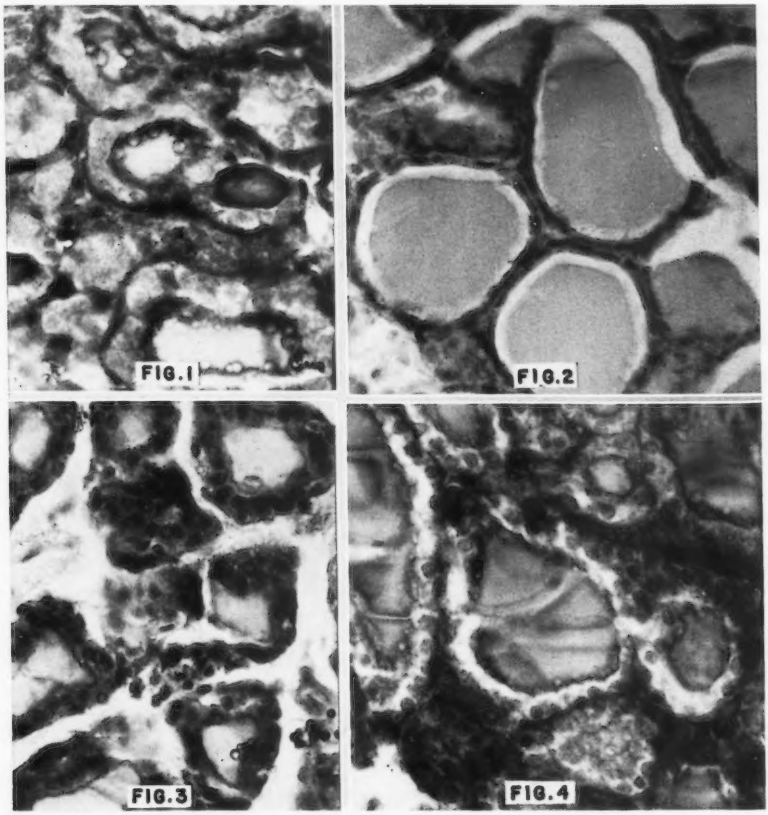
An ideal assay should include the following requirements. Three or 4 animals should be used in the assay of the biologic fluid in question. The totality of the pituitarectomy should be checked by gross palpation of the testes before starting the injections. (By the fifth postoperative day the testes should have shown a decrease in size and softening; the scrotum should have lost its normal turgescence and one or both of the testes should have ascended into the abdomen). At postmortem examination the pituitary fossa should be examined and sections made of any suspected material at the site of operation. Microscopic examination of the condition of the other endocrine glands such as the testis and adrenal should show atrophy. It is important to kill the animals at the end of the period of injections and to discard any which have died in order to obtain the microscopic material free of postmortem changes. These occur in the thyroid within 2 to 4 hours after death.

Selection of patients for assay. In this study only untreated and typical cases of thyrotoxicosis and myxedema were selected. Serum was obtained from venous blood drawn before any therapy was instituted. Urines were obtained as soon after voiding as possible and morning specimens were used in most instances. In addition to fluids obtained from 7 thyrotoxic patients and 9 myxedematous patients assays were carried out on the sera of 5 normal individuals and on the urines of 8 normal subjects.

Results of assays. The results of the assays can best be reported by reference to photomicrographs of histologic sections taken from typical examples of the assay material. Figure 1 shows a section of the thyroid gland of a normal (non-pituitarectomized) rat of our colony. The epithelium is cuboidal or low columnar. The colloid is abundant in amount, takes the stain uniformly and is slightly vacuolated at the edges. Figure 2 shows a section of a normal rat's thyroid 10 days after pituitarectomy. This rat received no injections and served as a control. This section shows the typical regression of the thyroid epithelium which takes place following pituitarectomy in the absence of any potent substitution therapy. Notable features of the section are the marked decrease in the height and number of epithelial cells, the large accumulation of colloid and the absence of even the normal degree of peripheral vacuolization of the uniformly stained acinar contents. Appearances entirely similar to these were obtained in the thyroid glands of totally pituitarectomized rats which had been treated with the bloods and urines of all of our normal individuals. Such assays will henceforth be referred to as 'negative for thyreotropic effect'. All the assays on bloods and urines of 8 cases of thyrotoxicosis gave similar negative findings.

Figure 3 is a photomicrograph of a section of thyroid gland from a pituitarectomized animal which received Collip's thyreotropic hormone from the fifth postoperative day for 5 days before sacrifice (0.25 cc. twice daily). This

section shows the characteristic 'support' of the thyroid histology by an adequate substitution therapy with thyreotropic material. This failure of regression of the thyroid epithelium following pituitarectomy will be referred to as evidence of a 'positive thyreotropic action' on the part of any material which produced it. The notable features of the section are the maintenance of the height and number of epithelial cells and the mild vacuolization of the colloid



which is not increased in amount and which shows a normal staining reaction.

Figure 4 shows a section of thyroid gland of an animal which was totally pituitarectomized 11 days before sacrifice and which received 5 cc. of urine from a completely myxedematous patient twice daily after the fifth postoperative day. The section shows an undoubtedly positive thyreotropic reaction. This is indicated by the presence of a mild degree of hyperplasia, columnar cells which are increased in number and show a slight tendency to papillary infolding, associated with marked vacuolization of the colloid which is decreased in amount and rather lightly stained. Entirely similar results were obtained with the urines of 8 and the blood sera of 9 myxedematous individ-

uals. A concentrate of 1 myxedematous patient's urine (1/5), made by vacuum distillation at room temperature gave a positive thyreotropic result whereas the unconcentrated urine gave a negative test. The assay on the serum of this patient was positive.

DISCUSSION

A comparison of figure 4 with figure 3 reveals the striking potency of myxedematous body fluids in supporting the thyroid histology following pituitarectomy as compared with the effect of the best available thyreotropic substance derived from pituitary tissue up to the time of these experiments. The appearance of slight hyperplasia in figure 4 is in sharp contrast to the regressive stage of the untreated pituitarectomized control animal's thyroid illustrated in figure 2. In contrast we believe we have demonstrated by the methods described above that the body fluids of neither normal nor thyrotoxic patients contain appreciable amounts of thyreotropic hormone in the quantities used in our experiments.

The fact that positive reactions were obtained with the body fluids of myxedematous patients indicates that the method is valid for the demonstration of the specific thyreotropic factor. The negative results obtained in normal and thyrotoxic patients serve as control data for the specificity of the reaction since neither the urinary contents nor the foreign serum protein of the negative samples had any effects on the thyroid histology of the pituitarectomized rat.

While this report confirms Aron's finding of 'thyreostimuline' in myxedema fluids we cannot agree that normal urine, even in amounts up to 90 cc. per rat, has any thyroid stimulating substance. We interpret Aron's positive results with normal urine as a reflection of the non-specificity of the guinea pig reaction since our substitutional assay method failed to confirm this. It is entirely conceivable, however, that Aron was able to detect the difference in titer of thyreotropic material which exists between myxedema and thyrotoxic fluids as shown by our results despite the non-specificity of his test method.

With the above data in mind we find it difficult to accept the anterior pituitary hypothesis in its present form as an explanation of the pathological mechanism of human Grave's disease despite the close analogy that has been drawn between the experimental and clinical syndromes. That the hyperthyroidism associated with anterior pituitary acidophilic tumors is on a pituitary thyreotropic basis seems likely. Cushing in 1927 found that of 72 cases of acromegaly more than half had a B.M.R. greater than + 10 per cent, the highest being + 61 per cent. Removal of a chromophil adenoma in his cases was followed by a "fall in B.M.R. as uniform and as striking as that which occurs following subtotal thyroidectomy in Grave's disease". However, it would appear from our data that there is no definite reason to suppose that the ordinary variety of patient with Grave's disease (not associated with acromegaly) is suffering from 'anterior pituitary hyperthyreotropism'.

That the urine and serum of cases of myxedema contain thyreotropic factor in considerable quantities deserves emphasis for several reasons. We believe this fact answers the question raised in the literature by Krogh and

Okkels as to whether thyreotropic material is excreted by the kidney. Furthermore the chemist will find in myxedema urine an excellent source of thyreotropic factor for purposes of purification and chemical identification.

SUMMARY

A method of substitutional assay is described as applicable to body fluids such as blood and urine for the detection of thyreotropic material. The method makes use of the pituitarectomized rat as test object and histologic examination of the thyroid glands of the assay animals is used in reading the test. Support of thyroid histology is considered as evidence of a positive test. Failure of injected material to prevent the regression of the thyroid epithelium following pituitarectomy is considered evidence of a negative test.

It was found by this method that blood serum and urine from myxedematous patients contain appreciable quantities of thyreotropic material, whereas none of the thyroid stimulating factor could be demonstrated in the body fluids of either thyrotoxic or normal individuals in the dosages used.

In discussion the current hypotheses of the mechanism of human Graves' disease are considered, and the significance to the chemist of the occurrence of thyreotropic factor in myxedema fluids is stressed. The specificity of the substitutional method of assay for the thyreotropic factor used in this study is held to be greater than that of other methods which have so far been used in attacking the problem.

REFERENCES

1. Rogowitch, N.: Beitr. z. path. Anat. u. zallg. Path. **4:** 453. 1888-9.
2. Niepce, P.: cited in Nice—med. **3:** 81. 1878-9.
3. Boyce, R. and C. F. Beadles: J. Path. & Bact. **1:** 223. 1892.
4. Wegelin, C.: Wien. klin. Wchnschr. **38:** 5. 1925.
5. Degener, Lyda M.: Quart. J. Exper. Physiol. **6:** 111. 1913.
6. Kojima, M.: Quart. J. Exper. Physiol. **11:** 255. 1917.
7. Adler, L.: Roux's Arch. **39:** 21. 1914.
8. Allen, B. M.: Science **44:** 755. 1916.
9. Smith, P. E.: Science **44:** 280. 1916.
10. Gudernatch, J. F.: Zentralbl. f. Physiol. **26:** 323. 1912.
11. Allen, B. M.: Anat. Rec. **20:** 192. 1921.
12. Smith, P. E. and J. P. Smith: Proc. Soc. Exper. Biol. & Med. **20:** 51. 1922.
13. Hogben, L. S.: Proc. Roy. Soc. London, S.B. **94:** 204. 1922.
14. Schwartzbach, S. S. and E. Uhlenhuth: Anat. Rec. **24:** 119. 1926.
15. Smith, P. E.: J.A.M.A. **88:** 158. 1927.
16. Aron, Max: Compt. rend. Soc. de biol. **102:** 682. 1929.
17. Siebert, W. J. and R. S. Smith: Proc. Soc. Exper. Biol. & Med. **27:** 622. 1930.
18. Schockaert, J. A.: Proc. Soc. Exper. Biol. & Med. **29:** 306. 1931.
19. Marine, D. and S. H. Rosen: Proc. Soc. Exper. Biol. & Med. **30:** 901. 1933.
20. Hertz, S. and A. Kranes: Endocrinology **18:** 415. 1934.
21. Friedgood, H. B.: Bull. Johns Hopkins Hosp. **54:** 48. 1934.
22. Friedgood, H. B.: Arch. Int. Med. **56:** 833. 1935.
23. Schockaert, J. A. and G. L. Foster: J. Biol. Chem. **95:** 89. 1932.
24. Marine, D.: J.A.M.A. **104:** 2250. 1935.
25. Aron, M., C. VanCaulaert and J. Stahl: Compt. rend. Soc. de biol. **107:** 64. 1931.
26. Krogh, M. and H. Okkels: Compt. rend. Soc. de biol. **113:** 635. 1933.

SIMILARITY OF THE IODIN REMISSION IN EXPERIMENTAL ANTERIOR HYPOPHYSEAL HYPERTHYROIDISM, THE HYPERTHYROIDISM OF ACROMEGALY AND THAT OF EXOPHTHALMIC GOITER

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Exophthalmic goiter is characterized by exophthalmos, an increased basal metabolic rate, tachycardia, parenchymal hypertrophy and hyperplasia of the thyroid gland with liquefaction and disappearance of its acinar colloid, nervous instability, an elevated blood iodin and a marked decrease in the iodin content of the thyroid gland. The symptomatic intensity of the malady may be marked either by remissions and exacerbations or a steady chronic state which is maintained for years. Iodids exert a remarkable, although temporary, depressant effect upon the course of the disease. In general, this description also applies well to the syndrome produced in guinea pigs by the daily intraperitoneal administration of an alkaline anterior hypophyseal extract. These similarities were pointed out in previous publications with certain reservations (1). It is proposed in the present investigation to analyze the data on the iodin remission from a similar viewpoint and to evaluate the possible relationship which may exist between the experimental syndrome and the clinical disease.

Dosage of Iodin in Relation to its Efficacy. Thompson and his associates (2) have made a systematic study of the effective range of iodin dosage in exophthalmic goiter. They found that the iodin remission is not an all-or-none phenomenon and that within certain limits larger doses exert a greater metabolism-depressant effect than small doses.

Similar studies were made in experimental anterior hypophyseal hyperthyroidism (3). In order to avoid confusion between the decrease in metabolic rate which occurs spontaneously (1) and the specific metabolism-depressant effect of iodin, the injections of sodium iodid were started simultaneously with those of the extract. These experiments showed that the effect of iodin was the same as in exophthalmic goiter (3). The response to a given dose ranged from one which was barely recognizable to one which completely obliterated the hyperthyroid state. Within the limits studied (0.0001 gm. to 0.2 gm. NaI per kg.), larger doses were more effective than smaller doses in preventing or partially interfering with the expected cycle of hyperthyroidism. The optimal depressant effect was elicited by iodin in doses approximating 0.084 gm. per kg. (0.1 gm. NaI).

Refractory Phenomenon in Iodin Therapy. All investigators are agreed

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TABLE 1. THE EFFECTIVE RANGE OF IODIN DOSAGE WHEN ADMINISTERED TO GUINEA PIGS SIMULTANEOUSLY WITH 2 CC. ANTERIOR PITUITARY EXTRACT PER KG. OF BODY WEIGHT.

Effect on B.M.R., Ordinarily Increased by A.P.E.	NaI per kg. Body Weight	17 Animals in Series (10♂, 7♀)	NaI per kg. Body Weight	18 Animals in Series (11♀, 7♂)
	Grams		Grams	
Complete or almost complete inhibition of increase	0.100 to 0.200	58.8% (7♂, 3♀)	0.0001 to 0.002	16.6% (1♂, 2♀)
Partial inhibition of increase	0.100 to 0.200	11.7% (1♂, 1♀)	0.0001 to 0.002	27.8% (2♂, 3♀)
No effect upon expected increase	0.100 to 0.200	29.5% (2♂, 3♀)	0.0001 to 0.002	44.4% (4♂, 6♀)

that iodin does not invariably induce a remission in the metabolic disturbance of exophthalmic goiter (4). Of the 434 cases included in these reports (4), 42 (10.7 per cent) failed to respond to iodid therapy. Means (5) states that in a recent study of over 500 cases only 4 per cent failed to show a decrease in metabolism after iodin therapy.

A similar initial refractoriness to iodids was found among a group of 35 guinea pigs in which experimental anterior hypophyseal hyperthyroidism was induced. Seventeen of these animals were given NaI in doses of 0.1 to 0.2 gm. per kg. of body weight; the other 18 received doses of 0.0001 to 0.002 gm. per kg. The percentage of cases which did not respond to iodin was greater among the 18 animals treated with relatively small doses of the drug (table 1).

Early Phase of the Iodin Remission. The time between the beginning of iodin therapy in exophthalmic goiter and the onset of a decrease in basal metabolism usually varies from 1 to 4 days (4h). The basal metabolic rate is

TABLE 2. THE IMMEDIATE EFFECT OF IODIN AND THE TRANSITORY DURATION OF ITS INFLUENCE IN EXPERIMENTAL ANTERIOR HYPOPHYSEAL HYPERTHYROIDISM.

No.	First Recorded Increase in B.M.R.		Maximum Recorded Increase in B.M.R. after Iodin Remission		Return to Normal B.M.R.		Secondary Decrease in B.M.R. (lowest level)		End of Experimental Period	
	%	Day	%	Day	%	Day	%	Day	%	Day
F	+24	14[4]	+24	14	+7	17	−8	21	−2	44
100	+10	6[3]	+22	13	—	—	—	—	+22	13
EE	+10	5[2]	+16	7–8	+5	14	−19	35	−10	43
101	+13	5[2]	+13	5–10	—	—	−6	13	−6	13
JJ	+11	2[1]	+21	6	+1	17	−7	23	+6	26
147	+18	11[5]	+18	11	+8 to 0	13 to 17	—	—	0	17
105	+19	4[3]	+19	4	+9	8	—	—	+9	8
NN	+10	3[2]	+18	6–12	—	—	—	—	+18	12
142	+13	3[1]	+26	12	—	—	—	—	+12	17
Mean	+14	6	+20	8	+6	14	—	—	—	—

[1] 1st determination.
[2] 2nd determination.
[3] 3rd determination.
[4] 4th determination.
[5] 5th determination.

generally found at its lowest level about the 8th to the 10th day, although the optimal effect may be delayed for 2 or 3 weeks (4a).

A similar variety of results (tables 2 and 3) were obtained in the experimental hyperthyroidism which ordinarily appears within 18 to 24 hours after the beginning of extract therapy (1). The effect of iodin upon this increased metabolic rate was found to be of two types, viz., immediate and delayed. The initial increase in metabolic rate was completely prevented in 15 of the 26 guinea pigs in which iodids were effective. In 10 others the depressant effects of iodin were delayed for 3 to 10 days; and in one the hyperthyroidism was unusually mild throughout the entire period of study.

TABLE 3. THE DELAYED EFFECT OF IODIN AND THE TRANSITORY DURATION OF ITS INFLUENCE IN EXPERIMENTAL ANTERIOR HYPOPHYSEAL HYPERTHYROIDISM IN 10 GUINEA PIGS.

No.	Maximum B.M.R. (Primary increase)		Iodin Remission (Lowest B.M.R.)		Secondary Increase in B.M.R. (Highest level)		Secondary Decrease in B.M.R. (First normal level)		Secondary Decrease in B.M.R. (Lowest level)		End of Experimental Period	
	%	Day	%	Day	%	Day	%	Day	%	Day	%	Day
G	+22	3†	+ 7	10	+20	14	+6	21	− 7	25	0	44
124	+16	2†	+ 3	4							+ 9	11
114	+12	1†	+ 7	3							+ 7	8
8B	+21	2†	+13	4	+39	8	+6	12			+ 3	21
DD	+18	2†	+ 5	7	+16	14	+1	16	− 6	23	− 6	44
B	+29	2†	+ 9	3	+32	12	+6	29			− 1	44
D	+23	2†	+17	9	+36	14	+6	16			+ 2	44
BB	+22	2†	+ 7	7	+19	12	−4	19	−15	28	− 6	44
140	+38	2†	+12	4	+58	13					+12	17
0	+31	5*	+16	8	+29	12					+29	12
Mean	+22	2	+10	6	+31	12	+4	19				

* Second determination. The primary increase in B.M.R. was delayed for 5 days, possibly because of iodin. The animal was included in this series because of a decrease in metabolic rate which took place after its initial increase.
† First determination.

Temporary Duration of the Iodin Remission. It is generally recognized that in most iodin-treated cases of exophthalmic goiter there is an optimal period during which the basal metabolic rate decreases to its lowest level, after which it increases in spite of the continued administration of the drug (4b, 4f, 6). This has led to the widespread practice of restricting the use of iodids in exophthalmic goiter to the preoperative preparation of patients and of performing the thyroidectomy sometime during the second or third week of therapy. Means (5) believes that this secondary increase in metabolism is not dependent upon refractoriness to iodin, but rather upon an increase in the degree of hyperthyroidism. As this writer has previously pointed out (1c), these two points of view are not necessarily irreconcilable. The administration of iodin is attended not only by a depressant effect upon the hyperthyroidism but also by the rapid restoration to the thyroid of a previously depleted store of hormone. The accumulation of this colloid in a gland which is in an unstable physiological state may result in the secretion of increasing amounts of thyroid hormone in spite of the depressant effects of iodids.

Escape from the depressant influence of iodin also occurs in experimental hyperthyroidism due to the thyreotropic principle (tables 2 and 3). The hyperthyroidism was completely suppressed during their entire period of study in 6 of the 15 animals showing an immediate iodin effect (table 2). In

the other 9 guinea pigs, the basal metabolic rate remained normal for only a brief period (2 to 13 days), after which the thyreotropic substance caused it to increase in spite of iodid administration. The metabolic rate increased at the beginning of the experiment in the 10 guinea pigs which were initially refractory to iodin (table 3). The eventual response to iodin in this series (table 3) was just as temporary as in the preceding one (table 2). The increase in metabolism which took place in both groups of animals after they became refractory to iodin was also of brief duration. After a few days, the metabolic rate returned to its normal level just as it does during the spontaneous remission. A third increase in metabolism, which ended similarly, was noted

TABLE 4. THE IMMEDIATE AND DELAYED EFFECTS OF NaI UPON THE THYREOTROPIC ACTIVITY OF AN ANTERIOR HYPOPHYSEAL EXTRACT.

Experimental Conditions	Sex	Experimental Behavior				
		Usual Cycle of Hyperthyroidism	Delayed Increase in Basal Metabolic Rate	Temporary Fall in Basal Metabolic Rate After its Initial Increase	Hyperthyroidism Generally Mild Throughout Experimental Period	Hyperthyroidism Completely Suppressed
		No. Animals	No. Animals	No. Animals	No. Animals	No. Animals
Control experiments with A.P.E. (25 animals)	M	12	2	2	0	0
	F	8	1	0	0	0
Total		20 (80%)	3 (12%)	2 (8%)	0	0
Experiments with A.P.E.+NaI (41 animals)	M	6	5	3	0	3
	F	9	4	7	1	3
Total		15 (36.5%)	*† 9 (21.9%)	*§ 10 (24.4%)	1 (2.4%)	6 (14.8%)

* Limited increase in basal metabolic rate at all times (5 males; 2 females).
† 5 guinea pigs.
§ 2 guinea pigs.

in one animal of the second group (table 3). In a number of guinea pigs, the rate of metabolism fell to unusually low levels after the completion of the spontaneous remission. The significance of this response will be discussed in a subsequent paragraph.

A small percentage of the animals in the control series (injected only with extract) were particularly resistant to the thyreotropic principle (table 4). These responses, being similar to those noted during a typical iodin remission, suggest some relationship between the mechanism of the latter and that of the natural immunity against the thyreotropic factor.

Exacerbations of Hyperthyroidism Due to Inadequate Dosage of Iodin. In their studies on exophthalmic goiter, Thompson and his associates (2) have found that occasionally the metabolic rate increases after treatment with small doses of iodin. The same thing happened in experimental anterior hypophyseal hyperthyroidism when animals were given sodium iodid in doses of 0.002 gm. or less per kg. of body weight (3). Twenty-five per cent of the animals

in the control series (injected only with extract) developed metabolic rates over +30 per cent between the 11th and the 15th days of experimentation; whereas over 38 per cent of those treated with extract plus small doses of sodium iodid reacted similarly. None of the animals which were injected with extract plus sodium iodid in doses of 0.1 to 0.2 gm. per kg. were found in this category.

Hypometabolism Resulting from Iodin Therapy. Haines (8) and Thompson and his associates (9) have described a group of patients with exophthalmic goiter in whom the administration of iodin causes a significant fall in basal metabolic rate to what are ordinarily considered moderate hypothyroid levels (seldom below —20 per cent). The latter occur with or without clinical evidence of hypofunction of the thyroid gland (myxedema). There are two types of cases showing this reaction, viz., one in which the subsequent rise to a normal rate of metabolism occurs while the patient is still receiving iodid therapy (temporary type) and the other in which this return to the normal metabolic level takes place only on omission of iodin (permanent type).

This phenomenon also occurs in animals receiving simultaneous injections of iodid and extract over a period of weeks (3). The behavior of the basal metabolic rate was studied for 21 days or more in 15 of 41 animals (3). In 11 of these the final readings ranged from +3 to —8 per cent of the original levels after the initial cycle of hyperthyroidism was entirely complete; 2 of these 11 animals also suffered a recurrent, mild transient period of hyperthyroidism (+12 and +20 per cent)after the spontaneous remission. In the remaining 4 of the 15 animals, the basal metabolic rate gradually fell to —10,[2] —15,[3] —18,[4] and —19[5] per cent, respectively, after the occurrence of the spontaneous remission. In 3 of these animals the metabolic rate was followed long enough to note a spontaneous return in the direction of the normal level, although the experimental therapy remained unchanged.

In earlier studies (1a, 1b) it was observed that iodin caused a sharp fall below normal in the metabolic rate if it were withheld until the beginning of the usual spontaneous remission. Subsequently (1c), 5 other animals were allowed to complete the entire cycle of hyperthyroidism before being injected with iodids in addition to extract. The metabolic rate fell to extremely low levels (comparable to those due to complete thyroidectomy in the guinea pig), but spontaneously returned to normal over a period of approximately 3 weeks in spite of unchanged experimental conditions. The dose of iodin capable of eliciting this response was extremely minute (0.0005 gm. NaI per kg.) as compared with that usually necessary to affect the rate of metabolism during the initial cycle of hyperthyroidism (0.1 gm. NaI per kg.).

As has been emphasized already (1c), the thyreotropic activity of the anterior hypophyseal extract used in these experiments and the metabolism-depressant effect of iodin are both of limited duration. For this reason, the

[2] Iodin did not affect the usual cycle of hyperthyroidism in this animal.
[3] See BB, table 3.
[4] Iodin completely obviated the occurrence of hyperthyroidism in this animal throughout its period of study.
[5] See EE, table 2.

changes in metabolism which result from their simultaneous administration depend on the duration and location of the period over which the transitory depressant effect of iodin impresses itself upon the equally transitory cycle of anterior hypophyseal hyperthyroidism. A significant increase in the rate of metabolism does not occur at any time during the experimental period if iodin suppresses the hyperthyroidism until the spontaneous remission develops. If the effective period of the iodin remission is shorter than the transient hyperthyroidism, the rate of oxygen consumption increases when the depressant activity of iodin disappears. Finally, the basal metabolic rate descends to hypothyroid or myxedema levels in certain guinea pigs when the depressant influence of the iodin extends beyond the period during which the anterior pituitary extract is capable of exerting its thyreotropic activity. This interpretation of the data would explain satisfactorily all of the results which have been obtained and would account also for those cases of exophthalmic goiter in which iodids produce not only an unusually prolonged remission, but also one which is accompanied by low levels of metabolism. In such patients the cycle of hyperthyroidism has probably approached close enough to the period of spontaneous remission to permit its over-lapping by the iodin remission. The occurrence of such a reaction during iodid administration may be of considerable practical importance in directing the therapy of patients with exophthalmic goiter .On the basis of the experimental results, one would be justified in predicting that such patients were entering a favorable phase of the malady and were more suitable for continued treatment with iodids rather than for thyroidectomy.

Effect of Iodin on the Hyperthyroidism of Acromegaly. The hyperthyroidism associated with early acromegaly is probably due to stimulation of the thyroid gland by an excess secretion of the anterior hypophyseal thyreotropic principle. It was therefore of interest to determine whether the administration of iodids in acromegaly would result in a significant decrease of the elevated rate of metabolism. In 1927, Cushing and Davidoff (10) reported a case of acromegaly in which iodin decreased the elevated basal metabolic rate just as it does in exophthalmic goiter. In 1930, the present writer studied a second case of acromegaly at the University Hospital in Ann Arbor, Michigan, with identical results under similar conditions. She was a middle-aged woman with typical acromegalic facies. Her history was irrelevant except for marked changes in her personal appearance. There was no objective evidence of hyperthyroidism except her elevated rate of metabolism and slight tachycardia, and her thyroid gland was palpable but not enlarged. Several x-ray examinations of her skull, during the interval of treatment with iodids (July, 1930), showed nothing except a marked thickening of both the inner and outer tables of the skull in the frontal region and an apparent nodular roughening of the inner table. Within the ensuing 2 years (July, 1932) her pituitary lesion had become enlarged sufficiently to cause erosion of the dorsum sellae.

The administration of Lugol's solution resulted in a significant decrease in both basal metabolic and pulse rates. The details of this response are recorded

in an abbreviated protocol which follows. It is noteworthy that the patient attended the Dispensary Clinic and was not confined to bed. She lived in Detroit but usually spent the night preceding the determination of her basal metabolism in Ann Arbor.

Date	B.M.R.	Pulse rate	Therapy	Experimental Period in days	Comment
5/ 2/30	+43	95	none		control
7/30/30	+39	92	none		control
7/30/30	—	—	Lugol's solution, 10 drops, twice daily	0	Lugol's solution started immediately after last B.M.R. determination
8/ 5/30	+38	92	Increase Lugol's solution to 15 drops, twice daily	6	
8/ 6/30	+32	80		7	
8/ 7/30	+25	76		8	
8/ 9/30	+24	80		10	
8/12/30	+27	80		13	

A third case of acromegaly with hyperthyroidism which responded favorably to iodin therapy has recently come to this writer's attention through the courtesy of Dr. J. H. Means. His patient had subjective symptoms and objective signs characteristic of hyperthyroidism in addition to acromegaly. The initial basal metabolic rate ranged from +30 to +45 per cent, with an average of +39 per cent on 4 successive determinations. The administration of iodin was followed by a progressive decrease in basal metabolic rate to a level of +18 per cent in 9 days, after which the rate of oxygen consumption returned to a level of approximately +30 per cent in spite of continued iodid therapy. The omission of iodin after the 20th day of the test period was followed within a few days by an increased rate of metabolism. This increase subsequently reached a level of +51 to +62 per cent by the end of the succeeding month.

DISCUSSION

The iodin remission in experimental anterior pituitary hyperthyroidism is comparable in all known respects with that observed in exophthalmic goiter. The administration of iodin to acromegalic patients with hyperthyroidism resulted in a similar depression of the elevated metabolic rate. This suggests the possibility that iodin affects the hyperthyroid state in these conditions by essentially the same mechanism, although the mode of stimulation of the thyroid may be quite different. As a matter of fact, the syndrome which develops in guinea pigs as a result of injections with an *alkaline extract* of the anterior hypophysis is not identical with exophthalmic goiter. Although it closely resembles this disease in many respects, there are also distinct differences between them which merit just as much emphasis as any of the similarities.

The hyperthyroid state in the experimental syndrome and that in exophthalmic goiter are similar in that they terminate spontaneously after a variable period of time. The anterior hypophyseal hyperthyroidism completes its course

regularly within a few weeks of its onset either in guinea pigs or man (1a, 1b, 1c, 12, 21) whereas in exophthalmic goiter the spells of hyperthyroidism are more prolonged (6 months to a year or more). The intrinsic mechanism of the remission in exophthalmic goiter is unknown, whereas in the experimental syndrome it is due in all probability to inactivation of the thyreotropic activity of beef extract through specific antibody formation (11, 1c). The earliest evidence for this belief is contained in a previous publication by the present writer (1a, 1b) who pointed out that recurrent increases in metabolic rate occurred after the initial spontaneous remission and that their onset coincided with a change in the batch of extract which was being injected. The reason for this secondary phase of hyperthyroidism was not apparent at that time. Werner (22), who has more recently confirmed and extended these observations, has been the first to supply a plausible explanation for this phenomenon. These experiments seem to establish the antibody nature of refractoriness to anterior hypophyseal thyreotropic substance.

The thyroid disturbance appears to be the same in anterior hypophyseal hyperthyroidism and exophthalmic goiter with reference to the elevated metabolic rate, the increased iodin content of the blood and the decreased concentration of iodin in the thyroid (1b). The histopathological changes in these thyroid glands are also comparable except in one important respect. The thyroids in both conditions appear to be similar with reference to hypertrophy and hyperplasia of the parenchymal cells, a marked decrease in the amount of acinar colloid and hypertrophy of the Golgi apparatus (1b). On the other hand, many investigators (13) have emphasized the characteristic occurrence of diffuse and circumscribed lymphoid infiltration of the thyroid gland in exophthalmic goiter. These have not been found in the thyroids activated by an anterior hypophyseal extract (1b).

The lymphoid infiltration of the thyroid gland is apparently only a part of the generalized lymphoid hyperplasia (regional lymph nodes, gastrointestinal tract, spleen, etc.) in exophthalmic goiter (13c, 13f, 13g, 14). This phase of the problem has not been investigated as yet in the experimental syndrome.

The thymus gland, which is commonly enlarged in exophthalmic goiter (13c, 13f, 13g, 14d, 15), has not been studied in mammals during a cycle of anterior hypophyseal hyperthyroidism; and Schockaert (16) reports that the thymus glands of ducks injected with such extracts are diminished in size as compared to those from normal control animals. Pathologists have found splenic enlargement in exophthalmic goiter (13c, 14d, 14f, 14i), and clinicians (14e, 14g, 14h) have reported palpable splenomegaly in 20 to 30 per cent of the cases. Statistical studies by the present writer (17) have shown that splenomegaly occurs in a small percentage of the guinea pigs developing hyperthyroidism after injections with an alkaline extract of the anterior hypophysis. Perla (18) has more recently confirmed these findings, using the rat as an experimental animal. Concurrent experimental studies on guinea pigs also revealed that the cortex of the adrenal glands (1a, 1b, 19) became significantly hypertrophied in a majority of the same hyperthyroid animals.

This is directly opposed to Warthin's (15f) findings in exophthalmic goiter, in which adrenal hypoplasia is the rule.

The data on exophthalmos likewise discloses that certain reservations must be made when comparing the experimental proptosis of guinea pigs with the exophthalmos characteristic of exophthalmic goiter. Some of the similarities between these two types of exophthalmos, with reference to the functional state of the thyroid gland, have already been pointed out in previous publications (1a, 1b); and in view of the comparative anatomical data it is surprising to what extent they do resemble each other. In the lower mammals there is a well-organized system of orbital smooth musculature which is mechanically in a position to cause protrusion of the eyeball (19). The acute exophthalmos which results from stimulation of the cervical sympathetics in such mammals is clearly due to the contraction of these smooth muscles, and presumably the provocative agent in the anterior hypophyseal extract also acts through this medium. On the other hand, these smooth muscles are said to be vestigial in man (20); and it is uncertain, from an anatomical point of view, to what one might theoretically attribute the clinical development of exophthalmos. According to Whitnall (20) and others, there is nothing grossly abnormal to be found in the orbital contents of patients with exophthalmos who die during an attack of acute exophthalmic goiter .

Some of these differences between the experimental and clinical conditions may or may not be due to the fact that a chemically crude extract of the anterior hypophysis was used throughout the experimental studies. This extract is known to contain thyreotropic, parathyreotropic, gonadotropic, adrenotropic and growth-promoting substances (1, 17). Enlargement of the spleen was also found during its administration in a small percentage of the animals (17). The simultaneous administration of so many active principles would obviously modify the experimental syndrome considerably. For this reason further investigation along these lines will be necessary when studies are made of the effects of highly purified anterior hypophyseal principles which are free of superfluous proteins and other impurities. For the present, at any rate, it would seem unjustifiable to attach any specific significance to the interesting parallelisms which have been disclosed. The current evidence does reveal, however, that the function of the thyroid gland may be profoundly altered by an extrathyroid agent. This finding may be construed as additional evidence for the belief that hyperthyroidism may be a symptom of exophthalmic goiter rather than its primary cause. A certain percentage of the cases of exophthalmic goiter may be traced eventually to a primary or secondary disturbance in the anterior hypophysis. This is a distinct possibility, because of the physiological relationship between the anterior hypophysis and the thyroid gland; but direct experimental proof of such a circumstance is still lacking.

SUMMARY

Forty-one guinea pigs were treated with sodium iodid during the course of hyperthyroidism produced by injections of an alkaline extract of the anterior hypophysis. The effect of iodin upon this hyperthyroidism was found to be

comparable in all known respects to its depressant action on the elevated metabolic rate in exophthalmic goiter. Iodids were also similarly effective in decreasing the elevated basal metabolic rate in the hyperthyroidism which is associated with early acromegaly.

The type of response to iodin therapy may be of prognostic value in certain instances of exophthalmic goiter. When iodids decrease the rate of metabolism to hypothyroid levels, the disease is probably entering a phase of remission in which it will respond favorably to prolonged iodin therapy.

The experimental syndrome resulting from daily administration of an alkaline anterior hypophyseal extract closely resembles exophthalmic goiter in some respects. There are, however, several points of difference between them, and the experimental condition has not been studied with reference to certain other important details. Judgment on the relationship between these two syndromes must be reserved until further investigations are carried out with more highly purified extracts of the anterior hypophysis.

REFERENCES

1. Friedgood, H. B.: (a) J.A.M.A. **100:** 521. 1933; (b) Bull. Johns Hopkins Hosp. **54:** 48. 1934; (c) Arch. Int. Med. **56:** 833. 1935.
2. Thompson, W. O., P. K. Thompson and A. C. Cohen: Arch. Int. Med. **49:** 199. 1932.
3. Friedgood, H. B.: (a) J. Pharmacol. & Exper. Therap. **51:** 134. 1934; (b) Ibid. **53:** 46. 1935.
4. (a) Plummer, H. S., and W. M. Boothby: Coll. Papers Mayo Clinic **15:** 565. 1923.
   (b) Starr, P., H. N. Segall and J. H. Means: Arch. Int. Med. **34:** 355. 1924.
   (c) Read, J. M.: Endocrinology **8:** 746. 1924.
   (d) Starr, P.: Arch. Int. Med. **39:** 520. 1927.
   (e) Holst, J., and G. Lunde: Am. J. Surg. **7:** 39. 1929.
   (f) Means, J. H., and E. P. Richardson: Oxford Monographs on Diagnosis and Treatment, Oxford University Press, New York **4:** 1929.
   (g) Coller, F. A., and E. B. Potter: Am. J. Surg. **6:** 609. 1929.
   (h) Thompson, W. O., A. G. Brailey, P. K. Thompson and E. G. Thorpe: Arch. Int. Med. **45:** 261. 1930.
5. Means, J. H.: Personal communication.
6. (a) Thompson, W. O., and P. K. Thompson: Arch. Int. Med. **48:** 351. 1931.
   (b) Means, J. H., W. O. Thompson and P. K. Thompson: Tr. A. Am. Physicians **43:** 146. 1928.
7. Means, J. H., and J. Lerman: J.A.M.A. **104:** 969. 1935.
8. Haines, S. F.: Endocrinology **12:** 55. 1928.
9. (a) Thompson, W. O., P. K. Thompson, A. G. Brailey and A. C. Cohen: Am. J. M. Sc. **179:** 733. 1930.
   (b) Thompson, W. O., and P. K. Thompson: J. Clin. Investigation **5:** 441. 1928.
   (c) Thompson, W. O., and P. K. Thompson: J. Clin. Investigation **5:** 471. 1928.
10. Cushing, H., and L. M. Davidoff: Arch. Int. Med. **39:** 673. 1927.
11. Smith, P. E.: J.A.M.A. **104:** 548. 1935.
12. Hertz, S., and A. Kranes: Endocrinology **18:** 415. 1934.
13. (a) Hezel, O.: Deutsche Ztschr. f. Nervenh. **4:** 353. 1893.
   (b) Farner, E.: Arch. f. path. Anat. **143:** 509. 1896.
   (c) MacCallum, W. G.: J.A.M.A. **49:** 1158. 1907.
   (d) Simmonds, M.: Deutsche med. Wchnschr. **37:** 2164. 1911.
   (e) Kocher, A.: Arch. f. path. Anat. **208:** 86. 1912.
   (f) Warthin, A. S.: Ann. Int. Med. **2:** 553. 1928; Detroit Proc. Inter-State Postgrad. Med. Assoc. No. Am., 1929, p. 383.
   (g) Simpson, W. M.; Am. J. Surg. **7:** 22. 1929.
14. (a) Barker, L. F.: J.A.M.A. **49:** 1235. 1907.
   (b) Kocher, A.: J.A.M.A. **49:** 1240. 1907.
   (c) Chvostek, V.: Morbus Basedowii und die Hyperthyreosen. J. Springer, Berlin, 1917.
   (d) Foster, N. B.: Nelson Loose-Leaf Living Medicine, Thos. Nelson & Sons, New York, **3:** 263. 1920.
   (e) Baldridge, C. W., and F. R. Peterson: J.A.M.A. **88:** 1701. 1927.
   (f) Delafield, F., and T. M. Prudden: A Textbook of Pathology. 14th Ed., Wm. Wood & Co., New York, 1927.

(g) Sainton, P., and G. Emond: Gaz. d. hôp. **103:** 449. 1930.
(h) Assmann, H.: München. med. Wchnschr. **78:** 221. 1931.
(i) Beattie, J. M., and W. E. C. Dickson: A Textbook of Special Pathology. Wm. Heineman, 3rd Ed. p. 841. London, 1926.
15. (a) Crotti, A.: Thyroid and Thymus. pp. 677-689. Lea & Febiger, Philadelphia, 1922.
(b) Moschcowitz, E.: Arch. Int. Med. **46:** 610. 1930.
16. Schockaert, J. A.: Arch. internat. de pharmacodyn. et de thérap. **41:** 23. 1931.
17. Friedgood, H. B.: (a) Medical Papers Dedicated to Henry A. Christian, Waverly Press, Baltimore, Feb., 1936; (b) Endocrinology **20:** 159. 1936.
18. Perla, D.: J. Exper. Med. **63:** 599. 1936.
19. (a) MacCallum, W. G., and W. B. Cornell: Med. News **85:** 732. 1904.
(b) Landström, J.: Ueber Morbus Basedowii, Thesis, Stockholm, 1907; reviewed in J.A.M.A. **52:** 476. 1909.
(c) Landström, J.: Exophthalmic Goiter According to Modern Views, Almänna svenka Läkaretidning, 1908, No. 48; abstracted in Ophthalmology, p. 675. 1909.
(d) Code, C. F., and H. E. Essex: Proc. Staff Meet., Mayo Clin. **10:** 808. 1935.
20. Whitnall, S. E.: The Anatomy of the Human Orbit and Accessory Organs of Vision, Henry Frowde and Hodder and Stoughton, London, 1921.
21. Thompson, W. O., S. G. Taylor, III, P. K. Thompson, S. B. Nadler and L. F. N. Dickie: Endocrinology **20:** 55. 1936.
22. Werner, S. C.: Proc. Soc. Exper. Biol. & Med. **34:** 390. 1936.

THE INFLUENCE OF PREGNANCY ON RESISTANCE TO THYROXINE, WITH DATA ON THE CREATINE CONTENT OF THE MATERNAL AND FETAL MYOCARDIUM

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Pregnant rats tolerate doses of thyroid and thyroxine which in non-pregnant controls cause rapid loss of weight and death. Indeed, pregnant rats thus treated may show increases of weight which, at least superficially, seem comparable to the gains of normal pregnant rats. This remarkable resistance was first observed in animals receiving desiccated thyroid, but in a more detailed study of the problem synthetic thyroxine (Hoffmann-LaRoche) has been used. The rats employed in this work were from a pure, inbred Wistar strain, reared in the laboratory under very favorable conditions. The diet was adequate in all respects and contained an overabundance of vitamin B (and G) as the requirement for this dietary essential seems to be considerably increased in hyperthyroidism (1), as well as in pregnancy.

The resistance of the pregnant rat to thyroxine is illustrated by the curves in figure 1. Rats 384, 385, 387, 388, 390, 391, 392, 393 and 394, previously virgin were mated on March 10, 1935. The mates were separated on the 13th day, at which time the administration of thyroxine (1 mg. per day, subcutaneously) was begun in the first 4 of these rats, this being continued until the day of delivery. The remaining 5 rats served as normal pregnant controls, while rat 386 was one of 5 non-pregnant controls receiving thyroxine. Rat 399 was an untreated, non-pregnant control.

Observations on a second group of rats, represented partly in figure 1, were commenced on April 8, 1935. As before, the mates were separated on the 13th day, when the daily injections were instituted. The curves for rats 420 and 422 typify the course of the thyroxine-treated animals of this series, while the curves for rats 418 and 419 represent the usual progress of the untreated pregnant and of the non-pregnant, thyroxine-treated rats, respectively.

During the early two-thirds of gestation the gain in weight of the pregnant rats was not much greater than that of the non-pregnant controls for a like period, but after the 14th day the gain in the former was very rapid. In

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the pregnant rats receiving thyroxine there was at first a moderate loss of weight, or a diminished rate of increase, followed in 3 or 4 days by a gain in weight almost comparable to that of the untreated pregnant rats. In those animals which delivered during the night, the previous recorded weight was usually obtained 12 to 20 hours before labor. The maximum weight, therefore, may have been somewhat above that indicated by the curves. All of the non-pregnant, thyroxine-treated controls showed a progressive loss of

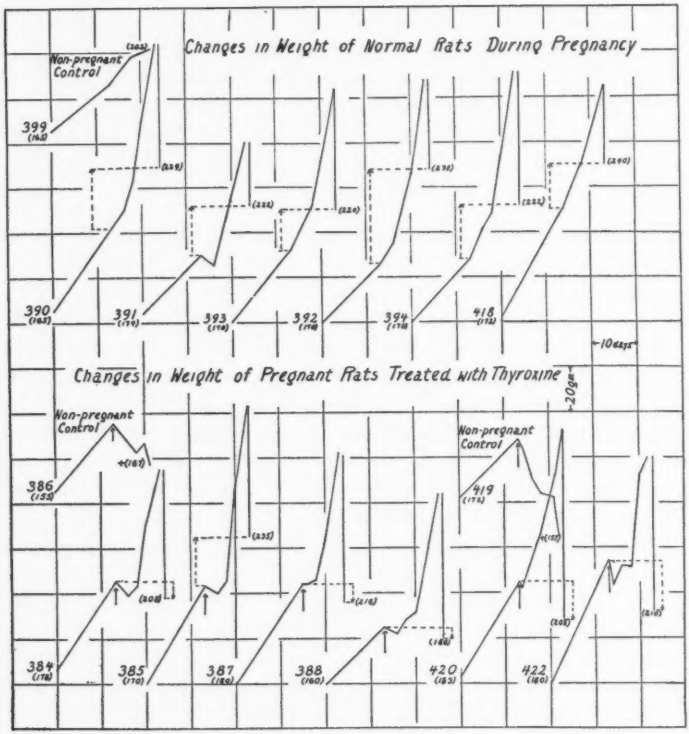

Fig. 1. Weight curves of pregnant rats treated with thyroxine and of their non-pregnant and untreated, pregnant controls. The numbers in parentheses denote the initial and final weights.

weight, several of the animals succumbing before the conclusion of the experiments.

The gestation period for the rat is normally 21.5 to 22 days (2). Two of the 6 untreated rats, represented in figure 1, delivered on the 23d day, the rest by the 24th day. Three of the thyroxine-treated rats delivered on the 23d day, 2 on the 24th, and 1 on the 26th day. As no attempt was made to fix the actual time of insemination in these and a larger number of similar experiments, it is not possible to state with certainty whether the period of gestation was actually prolonged in any of the treated rats. However a sufficient number of these delivered within the normal period of 22 days to sug-

gest that while hyperthyroidism in the rat may often be associated with prolonged gestation, this is by no means a consistent finding (compare with Gudernatsch, 3).

Without exception the normal pregnant controls in the 2 series under consideration weighed more after delivery than 10-11 days previously when the mates were separated, the gain varying from 18 gm. for rat 392 to 44 gm. for rat 394 (average gain 26 gm.). Only 1 of the thyroxine-treated pregnant

TABLE 1. CREATINE CONCENTRATION OF THE MYOCARDIUM AND SKELETAL MUSCLE OF THE MOTHERS AND OF THE MYOCARDIUM OF THE OFFSPRING, WITH DATA CONCERNING THE LITTERS OF THE THYROXINE-TREATED RATS AND THEIR NORMAL CONTROLS.

Rat No.	Number in litter		Weight of Litter, Gm.	Creatine Concentration Mg. per 100 gm. of Tissue		
	Living	Still-born		Muscle (maternal)	Myocardium (maternal)	Myocardium (new-born)
Normal Pregnant Rats						
390	12	0	62.1			87.6
391	7	2	42.5	525	226	82.5
392	7	0	37.2	518	189	98.5
393	10	0	55.6			82.7
394	9	0	57.0	523	196	75.2
418	5	2	36.0	497	188	85.5
Average	9.0		48.4	516	200	85.4
Thyroxine-treated Pregnant Rats						
384	6	2	40.8	461	138.5	73.7
385	7	5	48.0	471	135.5	92.4
387	11	1	58.2	480	135.9	72.4
388		6	23.6	404	134.5	
420	8	4	36.5	473	116	80.6
Average	9.83		44.2	465	127.9	82.4
Thyroxine-treated, Non-pregnant Rats						
383				398	121	
386				419	113.7	
396				462	123	
398				470	126	
419				418	93.2	
Average				433	115.4	

rats showed an absolute increase in weight (22 gm.), the others weighing somewhat less after delivery than before the administration of thyroxine was begun.

Because of the effect of hyperthyroidism in diminishing the concentration of creatine in muscle, especially of the myocardium (4, 5, 6, 7), it seemed desirable to investigate the changes, if any, of the maternal and new-born organisms. The method of Rose, Helmer and Chanutin (8) for tissue creatine was used. As indicated by the data in table 1, pregnancy itself did not influence the creatine level in either skeletal or cardiac muscle, the values obtained being normal. In the thyroxine-treated group the creatine concentra-

tion of the voluntary muscle was only moderately diminished, the depletion being much more marked in the heart. From the limited number of observations at hand, it would seem, however, that these changes were perhaps somewhat less pronounced than in the hyperthyroid non-pregnant rats.

Hyperthyroidism in the mother produced no demonstrable effect on the creatine content of the fetal hearts. Values of 75.2 to 98.5 mg. per 100 gm. (average 85.4 mg.) were obtained in the new-born of the untreated group, while in the new-born of the thyroxine-treated rats the values were from 72.4 to 93 mg. (average 82.4 mg.). It is interesting to note that similar relations hold in man, the creatine concentrations of the myocardium of the newborn and of the adult being comparable to the values observed in the rat (unpublished data).

From the illustrative data recorded in table 1, it will be seen that in the normal rats the average number per litter was 9 and the average weight, 48.4 gm. In the thyroxine-treated group the number per litter averaged 9.83 and the weight 44.2 gm. The striking difference was that of a total of 54 offspring in the control group, there were only four still-born, whereas of the 59 in the thyroxine-treated group, 23 were either still-born or died within an hour after birth.

SUMMARY

Pregnant rats have been found to possess a remarkable tolerance to thyroxine. Despite the administration, subcutaneously, of 1 mg. daily during the last 10-12 days of gestation such rats showed almost the same gain in weight as their untreated pregnant controls. However, a comparison of the weights before the thyroxine treatment was instituted and at the end of labor revealed a moderate loss in most cases, in contrast to the usual, absolute gain in weight of the control rats. The non-pregnant, thyroxine-treated controls invariably lost weight.

In a considerable proportion of the thyroxine-treated rats the gestation period was normal. The number per litter was approximately the same but the weight was somewhat less in the thyrotoxic group. A much greater incidence of still-births occurred in the thyroxine-treated rats than in the normal controls.

Although hyperthyroidism in the mothers produced a marked diminution of the creatine content of the myocardium and cardiac hypertrophy, no similar effects were produced on the fetal hearts, the size and creatine concentration being within normal limits.

REFERENCES

1. Sure, B., and M. E. Smith: J. Nutrition. **7:** 547. 1934.
2. Donaldson, H. H.: The Rat, Memoirs of the Wistar Institute of Anatomy and Biology, Philadelphia, 2nd edition, p. 20. 1924.
3. Gudernatsch, J. F.: Am. J. Physiol. **36:** 370. 1914.
4. Cowan, W. D.: Am. J. Physiol. **109:** 312. 1934.
5. Bodansky, M. and J. F. Pilcher: Proc. Soc. Exper. Biol. & Med. **32:** 597. 1935.
6. Bodansky, M.: J. Biol. Chem. **109:** 615. 1935.
7. Bodansky, M., J. F. Pilcher and V. B. Duff: J. Exper. Med. **63:** 523. 1936.
8. Rose, W. C., O. M. Helmer and A. Chanutin: J. Biol. Chem. **75:** 534. 1927.

AGE AS A FACTOR IN THE RESISTANCE OF THE ALBINO RAT TO THYROXINE, WITH FURTHER OBSERVATIONS ON THE CREATINE CONTENT OF TISSUES IN EXPERIMENTAL HYPERTHYROIDISM

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Loss of weight is a well recognized effect of thyroid feeding, especially in mature animals. In the young the results have been somewhat at variance, definite acceleration to marked retardation of growth and even loss of weight having been reported by different investigators (1-7). This is not surprising in view of the various kinds of thyroid preparation that have been used, the discrepancy in dosage and other divergent experimental conditions. Considered in the light of present knowledge, the diet was probably inadequate in certain of the earlier studies.

Of particular interest is the work of Cameron and Carmichael (7). These investigators adjusted the daily dose of thyroid to a fixed proportion of the body weight and found that growth was somewhat depressed as the result of the continued administration of desiccated thyroid in amounts as small as 1:20,000. When the dose was increased to 1 part per 5000 parts of body weight retardation was much more pronounced, amounting in some cases to complete cessation of growth. Similar results were obtained by Cameron and Carmichael (8) with extremely small doses of thyroxine added daily to the food.

A comparison of the effects observed by Cameron and Carmichael (9) on feeding small doses of thyroid (1:5000) to young rats, with those obtained by Cameron and Sedziac (10) in adult rats, reveals that while the animals in the latter group invariably lost weight, the less mature ones in the first group usually gained weight, although not at the same rate as the normal controls. This is suggestive of a somewhat greater tolerance of the immature rats to thyroid, a point not particularly stressed, however, by the authors. Indeed from a survey of the extensive literature on the physiology and pharmacology of the thyroid hormone, it would seem that the extraordinary resistance shown by young animals is not fully appreciated.

In a preliminary series of experiments it was noted that the continued administration of desiccated thyroid (Lilly), in daily doses of 250 mg. per

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100 gm. of body weight (this is more than ten times the maximum dosage used by Cameron and Carmichael, 8), was much less toxic to young rats than to adult rats. Since the dosage could be better controlled by the subcutaneous injection of thyroxine, the synthetic preparation of Hoffmann-LaRoche was used in the more detailed study of the problem. It is the purpose of this paper to demonstrate an unsuspected degree of tolerance of immature rats to thyroxine.

The rats used were from a pure inbred Wistar strain reared in the labora-

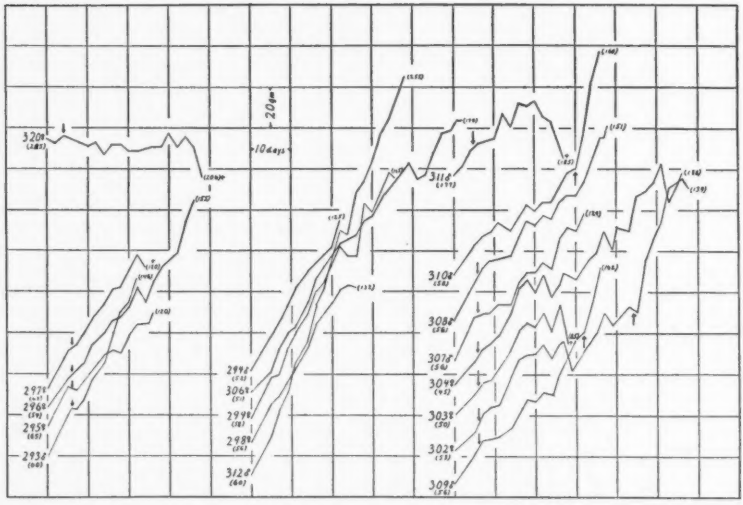

Fig. 1. Showing weight curves of thyroxine-treated young rats and their adult and normal litter-mate controls (294, 298, 299, 306, 312). Rats 293-299 were born Jan. 1. Rats 302-312 were born Jan. 2. Observations were begun Jan. 30 and the injections of thyroxine were started 6 days later, as indicated by downward pointing arrows. Rat 311 belonging to the 302-312 litter received the first injection of thyroxine at 59 days of age (March 1). Rat 320 was the adult, thyroxine-treated control. Cessation of injections in several subjects (302, 309, 310) is indicated by upward pointing arrows. The numbers in parentheses denote the initial and final weights.

tory under favorable conditions. The basic diet consisted of Purina Chow, a dog food preparation, supplemented by a mixture composed of ground Chow, 2 parts, a dried milk preparation, 1 part, brewers' yeast 0.1 part, and cod-liver oil. The supplementary ration was furnished in liberal amounts to the mothers during the second half of pregnancy as well as during the entire lactation period; to the young before and for some time after weaning, and to all animals throughout the experimental period. In this way an overabundance of vitamin B and other essentials of nutrition was assured. According to Sure and Smith (11), the vitamin B requirement is increased in hyperthyroidism, an observation fully supported by the experience in this laboratory.

The environmental temperature materially influences the resistance of rats to thyroid and thyroxine, as shown elsewhere (12). As it would have been impractical to keep the animals at constant temperature for the long periods required in this work, the observations were conducted throughout the year,

a record being available of the extreme and mean daily temperatures. Two series of experiments are presented in this paper. In figure 1 are charted the results of experiments begun Jan. 30, 1935 and continued for a variable period up to nearly 60 days. The experiments represented in figure 2 were commenced Oct. 11, 1935.

Because of the moderate external temperature (15-22°C) during the experimental period represented by figure 1, the weight of the adult control (rat 320) was maintained comparatively well on a daily dose of 1 mg. of thy-

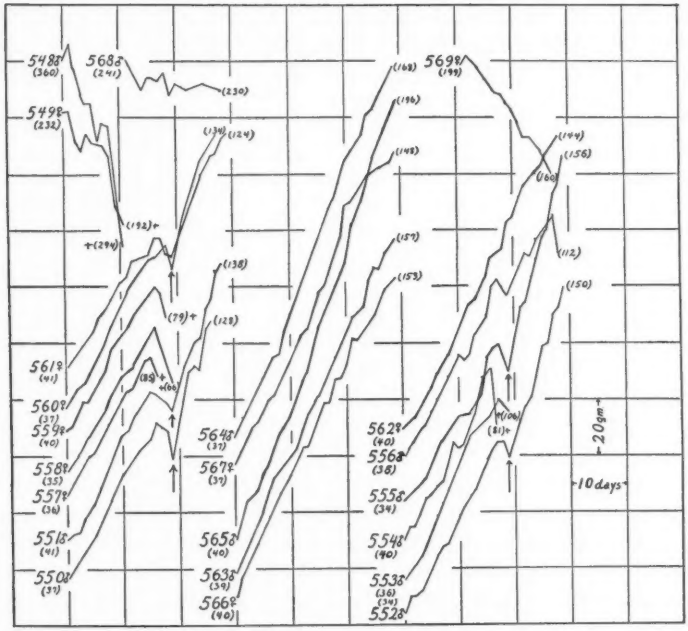

Fig. 2. Showing weight curves of thyroxine-treated rats and their litter mate controls (563-567, incl.). Two litters are represented. Rats 550, 551, 557, 558, 559, 560, 561, 563 and 566 were 27 days old at the beginning of the experiment; the rest were only 22 days old. Rats 548 and 549 were the first pair of adult controls. When these succumbed, 568 and 569 were started. Cessation of thyroxine injections is indicated by upward pointing arrows (550, 551, 552, 555, 561). The initial and final weights are designated by the numbers in parentheses.

roxine. The young in this group, weighing approximately one-fourth of the control but receiving the same amount of thyroxine, invariably gained weight, though at a subnormal rate. When the administration of thyroxine was discontinued, as indicated by the upward pointing arrows, growth followed at an accelerated rate, an effect that has been previously described by Cameron and Carmichael (13).

The difference in tolerance is even better illustrated in figure 2, in which are charted the results on 2 litters of rats, aged 22 and 27 days, respectively, at the beginning of the experiment. These animals gained weight at a fairly rapid rate and only a few succumbed (553, 557, 558, 559), while the first

pair of adult controls, weighing nearly 10 times as much and receiving precisely the same dose of thyroxine lost weight rapidly and died on the 11th day of the experiment.

In young, as in old hyperthyroid rats, the myocardium suffers a marked reduction of its creatine content (14) but apparently the former withstand losses of 40 to 50 per cent better than the latter. While a satisfactory explanation of this difference in resistance cannot be offered at this time, it may be suggested that the more prompt and effective cardiac hypertrophy and compensation in the younger individuals may be an important factor.

A statistical summary of the data obtained on analyses of muscle and heart of 17 thyroxine-treated and 8 control rats is given in the accompanying table. The values for P, calculated by the method of Fisher (15), indicate that

	Creatine in Ventricle Mg./100 gm.		Ratio, Ventricular Wt./body Wt. Gm./100 gm.		Creatine in Skeletal Muscle Mg./100 gm.	
	Mean and σ_m	St.D. of Distribution	Mean and σ_m	St.D. of Distribution	Mean and σ_m	St.D. of Distribution
17 thyroxine-treated rats	107±2.1	± 8.3	0.66±.032	±0.130	403±6.8	±27.3
8 Normal controls	185±9.7	±26.2	0.32±.008	±0.022	460±6.7	±18.1
Difference 'P' (Fisher)	78±9.9 <.01		0.34±.033 <.01		57±9.5 < .01	

the chances are less than 1 in 100 that the administration of thyroxine does not produce a real difference in the creatine concentration of heart and skeletal muscle and in the ventricular weight, body weight ratio.

When the thyrotoxic rats were permitted to recover by discontinuing the administration of thyroxine, the heart creatine values returned to normal within 7 to 14 days but, as the hearts remained oversize much longer, the actual creatine reserve per heart attained a value considerably above normal. Restitution of the creatine content in skeletal muscle occurred more gradually than in the heart. In the limited number of analyses, no significant difference was found in the creatine content of the testes of mature and immature rats. In both groups the concentration was somewhat increased in hyperthyroidism, this being in marked contrast to the depletion in skeletal and cardiac muscle.

Referring again to the tolerance of the immature rats to thyroxine, it would seem from the data presented that the growth impulse definitely overbalanced at least some of the effects associated with hyperthyroidism in the adult. The continued gain in weight of the young rats, compared with the loss in the grown animals receiving proportionately much smaller amounts of thyroxine, indicates a qualitative, if not a quantitative, difference in the interplay of forces resulting from an oversupply of thyroid hormone. The recognized interrelationships of the thyroid, pituitary, thymus and gonads suggest a possible approach to the further study of the problem.

SUMMARY

Immature rats have been shown to possess a remarkable tolerance to thyroxine. Rats 22 to 36 days old at the beginning of treatment continued to grow, though at a subnormal rate, on doses of thyroxine (1 mg. daily, subcutaneously) which in adult animals, weighing 5 to 10 times as much, caused rapid loss of weight and death.

In young, as in old, hyperthyroid rats, the myocardium suffers a marked reduction of its creatine concentration but the former apparently withstand losses of 40 to 50 per cent better than the latter. In part this may be due to more prompt and effective cardiac hypertrophy and compensation occurring in the younger individuals. As is the case in mature rats, hyperthyroidism in the young is associated with a diminution in the creatine content of skeletal muscle and an increase in the testes.

When the administration of thyroxine is discontinued, the creatine concentration of the ventricles is restored to normal in about 7 to 14 days. On the other hand, such reduction as may occur in the size of the heart takes place much more slowly.

Cessation of the treatment with thyroxine in the young animals was followed immediately by an acceleration of growth at a rate above normal.

REFERENCES

1. Schäfer, E. A.: Quart. J. Exper. Physiol. **5:** 203. 1912.
2. Iscovesco, H.: Compt. Rend. Soc. de biol. **75:** 361. 1913.
3. Gudernatsch, J. F.: Am. J. Physiol. **36:** 370. 1915.
4. Hoskins, E. R.: J. Exper. Zool. **21:** 295. 1916.
5. Herring, P. T.: J. Exper. Med. **11:** 231. 1917.
6. Smith, P. E., C. F. Greenwood and G. L. Foster: Am. J. Path. **3:** 669. 1927.
7. Cameron, A. T., and J. Carmichael: J. Biol. Chem. **45:** 69. 1920.
8. ————: J. Biol. Chem. **46:** 35. 1921.
9. ————: Am. J. Physiol. **58:** 1. 1921.
10. ————: and F. A. Sedziak: Am. J. Physiol. **58:** 7. 1921.
11. Sure, B., and M. E. Smith: J. Nutrition. **7:** 547. 1934.
12. Bodansky, M., J. F. Pilcher and V. B. Duff: J. Exper. Med. **63:** 523. 1936.
13. Cameron, A. T., and J. Carmichael: Trans. Roy. Soc. Canada. **16,** Sect. V: 57. 1922.
14. Bodansky, M. J. Biol. Chem. **109:** 615. 1935.
15. Fisher, R. A., Statistical Methods for Research Workers, 4th edition, Sect. 24.1, Oliver and Boyd, Edinburgh, 1932.

ANALYSES OF URINE OF THE CHIMPANZEE FOR ESTROGENIC CONTENT DURING VARIOUS STAGES OF THE MENSTRUAL CYCLE

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The urine of pregnant women and mares is an abundant source of both estrogenic and gonadotropic substances (1, 2). Some other mammals excrete relatively little during pregnancy (3). Recovery of considerable amounts of estrogenic material from the urine of pregnant chimpanzees has been reported in an earlier paper (4). Yields ranging from 170 to 620 mouse units per liter of urine were obtained at various stages of pregnancy. Chimpanzee placentas also proved to be a good source of estrogenic material, being similar to the human in this respect. At the same time Zuckerman (5) reported the diagnosis of pregnancy by series of Aschheim-Zondek tests for anterior pituitary-like hormone in urine from these pregnant chimpanzees.

In recent years perfection of extraction methods has made possible assays of small amounts of excreted estrogenic materials in the urine of non-pregnant animals. Of greatest interest has been the variation in the amount excreted at different times in the menstrual cycle in women (6, 7, 8, 9, 10, 11).

The female chimpanzee has a menstrual cycle similar to that of woman and in addition has, as a striking secondary sex character, an extensive swelling involving the external genital organs and surrounding areas (12, 13). This swelling begins a few days after menstruation, increases to a maximum in 10 to 15 days and ordinarily remains in this condition for about 10 days. It then retrogresses, sometimes quite suddenly, before the onset of the next menses. In some respects this genital swelling of the female chimpanzee is similar to the swelling of the 'sexual skin' of monkeys (14). It seems to be due primarily to the transformation of the subcutaneous connective tissue which involves a temporary accumulation of intercellular material (15).

The reddening and swelling of the 'sexual skin' has been produced experimentally in ovariectomized monkeys by injections of estrogenic material (16, 17), while long continued injections of massive doses (18, 19) have produced extreme conditions. In chimpanzees then, as in monkeys, this swelling of the 'sexual skin' probably serves as a good indicator of the concentration of estrogenic material in the blood.

In the human female the entire quantity of estrogenic material excreted during one complete menstrual cycle may be as much as 4000 m.u. (8).

[1] This work was supported by grants to Drs. Allen and Yerkes from the Committee for Research in Problems of Sex of the National Research Council.

Marked variations occur but in general it may be said that excretion rises during the follicular phase of the cycle and reaches a first peak at about the midpoint between the onset of 2 successive menstrual periods (the approximate time of ovulation). A second peak of excretion may sometimes occur in women during the week preceding onset of menses. Gustavson and Green (10) found highest yields from the 9th to 12th and the 14th to 21st days. This has been interpreted as indicating that growing follicles and fully developed corpora lutea are responsible for the increased excretion. Since high concentrations of

ASSAYS OF ESTROGENIC MATERIAL EXCRETED AT DIFFERENT STAGES OF THE MENSTRUAL CYCLE IN THE CHIMPANZEE

Animal	Date of Collection	Day of Menstrual Cycle	Notes on Menses and Genital Swelling*	Quantity of Urine Sample (cc.)	Mouse Units of Estrogen	
					8 hours	24 hours
Lia	Aug. 9	1st	Menstruation	1060		60
Nina	July 31	2nd	Menstruation	1110		49
	Sept. 7	5th	Menstruation	820		53
	Sept. 18	16th	+¾	1100		88
Pati	July 19	14th	Maximum swelling	1670		120
	Aug. 8	1st	Menstruation (no swelling)	1680		91
	Aug. 13	6th	No swelling	1250		67
	Aug. 17	10th	+½	1350		102
	Aug. 24	17th	−¾	1530		142
	Sept. 14	7th	+¼	1350		70
	Sept. 20	13th	Maximum swelling	1660		126
	May 22	18th	Maximum swelling	1030	39	
	May 29	25th	No swelling	1040	88	
	June 5	2nd	Menstruation	910	37	
	June 9	6th	No swelling	505	56	
	June 14	11th	+¼	980	48	
	June 21	18th	Maximum swelling	964	100	
Wendy	May 26	14th	Maximum swelling	565	66	
	May 30	18th	Day detumescence began	1110	55	
	June 4	23d	No swelling	740	76	
	June 12	2nd	Menstruation	276	9	
	June 15	5th	+⅛	734	78	

* The fractions in column 4 refer to the degree of swelling; + signs indicate that the swelling was increasing, — signs, decreasing.

theelin have been demonstrated in both of these structures in the human ovary (20), these results are confirmatory.

In the chimpanzee there is a rather sharp decline in the genital swelling a week or so before the onset of menses, which in itself might indicate a rapid drop of concentration of this hormone in the blood stream. It seemed highly desirable, therefore, to make quantitative assays of amounts excreted in nonpregnant chimpanzees for comparison with those of women.

Preliminary studies with 2 females throughout 2 complete cycles indicated the desirability of concentrating attention on certain critical periods within the cycle. Most of our subsequent tests were made with specimens from the periods of bleeding, the first days of genital swelling, maximum swelling, and during the early phase of detumescence. Complete daily records of the sexual status

of all females in the Yale Laboratories of Primate Biology constitute the data for correlation with the various amounts of estrogenic hormone excreted.

Urine samples were collected in a specially arranged outdoor cage which, in warm weather, provided fairly comfortable quarters for an animal even for 24-hour periods. Samples were shipped promptly to New Haven where all extractions were made.

The first collections were made for periods of 8 hours. The daily time of collection, the water intake, and other conditions were standardized as much as possible to make the yields of excreted hormone comparable for different stages of the menstrual cycle. Later 24-hour collections were undertaken for 2 animals. A total of 48 samples of urine from 5 chimpanzees were extracted and assayed in more than 340 tests in ovariectomized mice. The criterion of activity was the complete estrous growth reaction of the vaginal epithelium as judged by smear preparations.

METHODS OF EXTRACTION AND ASSAY

The method of extraction was that used by Curtis[2] in similar work on urine from non-pregnant women. Urine specimens were allowed to putrify at room temperature as a preliminary to extraction. Ten days was set as the standard time. Then sufficient hydrochloric or sulphuric acid was added to make the solution acid to Congo red. Five cc. of casein plus 10 cc. of sodium tungstate were added to each 500 cc. of urine. The solution was reacidified, stirred for 15 minutes, and then centrifuged. The residue was washed 3 times with acetone. The acetone washings were evaporated and the residue taken up in 0.5 per cent sodium hydroxide. The final product was neutralized to the basic side of phenophthalein with acetic acid.

The results as shown in the table indicate the yields from 24 and 8-hour samples. All of the samples returned positive results. When collections were made over 24-hour periods the yields ranged from 49 to 142 M.U. per sample. When collections were made for 8-hour periods the range was from 9 to 100 M.U. per sample. The highest yields were obtained when the genital swelling was present and recorded from one-half to maximum, during and just following the follicular phase of the menstrual cycle. The lowest yields were obtained from collections during menstruation. Thus the amount of estrogenic substance excreted clearly parallels the degree of genital swelling.

SUMMARY

Assays have been made of urine from non-pregnant chimpanzees at various stages of the menstrual cycle. Considerable amounts are excreted. Greatest yields were obtained at the time of maximum genital swelling; i.e., between onset of 2 menstrual periods. Lowest yields were obtained from urine collected during menstruation.

REFERENCES

1. Aschheim, S., and B. Zondek.: Klin. Wchnschr. **6**: 1322. 1927.
2. Aschheim, S.: Chap. XVII. Glandular Physiology and Therapy. A.M.A. Press, Chicago, 1935.

[2] Acknowledgment is made to Dr. Jack M. Curtis, National Research Council Fellow, working as biochemist on endocrine problems in the Department of Anatomy.

3. Engle, E. T.: Chap. XVI, Sex and Internal Secretions, Williams & Wilkins, Baltimore, 1932.
4. Allen, E., A. W. Diddle and J. H. Elder: Am. J. Physiol. **110:** 593, 1935.
5. Zuckerman, S.: Am. J. Physiol. **110:** 597. 1935.
6. Loewe, S., and F. Lange: Klin. Wchnschr. **5:** 1038. 1926.
7. Frank, R. T., and M. A. Goldberger: J.A.M.A. **94:** 1197. 1930.
8. Siebke, H.: Zentralbl. f. Gynäk. **54:** 1618. 1930.
9. Kurzrok, R.: Endocrinology **16:** 361. 1932.
10. Gustavson, R. G., and D. F. Green: J. Biol. Chem. **105:** xxxiv. 1934.
11. Frank, R. T.: Chap. XVI, Glandular Physiology and Therapy. A.M.A. Press, Chicago, 1935.
12. Tinklepaugh, O. L.: J. Morphol. **54:** 521. 1933.
13. Yerkes, R. M., and J. H. Elder: Proc. Nat. Acad. Sc. (In press).
14. Corner, G. W.: Contrib. to Embryol. No. 75, Carnegie Inst. Wash. **15:** 73. 1923.
15. Collings, M. R.: Anat. Rec. **33:** 271. 1926.
16. Allen, E.: Contrib. to Embryol., Carnegie Inst. Wash. **19:** 1. 1927.
17. Allen, E.: J. Morph. & Physiol. **46:** 479. 1928.
18. Bachman, C., J. B. Collip and H. Selye: Proc. Roy. Soc., London, S.B. **117:** 16. 1935.
19. Bachman, C., J. B. Collip and H. Selye: Proc. Soc. Exper. Biol. & Med. **33:** 549. 1936.
20. Allen, E., J. P. Pratt and E. A. Doisy: J.A.M.A. **85:** 399. 1925.

THE RÔLE OF THE CORPORA LUTEA IN PROLONGING THE LIFE OF ADRENALECTOMIZED RATS

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The life-sparing action of the ovaries in adrenalectomized animals has been noted in several instances in dogs (1, 2) and in cats (3). Stewart (4) thinks the mechanism concerned in these cases is in the corpora lutea and interstitial cells of the ovaries. The recent trend in endocrinology seems to place most of our glands of internal secretion under control of, or at least modified by, hormones or substances secreted by the hypophysis. For example, the ovaries (5), the testes (6), the thyroid (7), the parathyroids (8), the adrenals (9) and the pancreas (10) have been shown to be dependent for normal function upon the secretions from the pituitary gland. With these facts in mind, we have devised experiments to show that the pituitary gland, through the ovary, is capable of prolonging the life of adrenalectomized rats.

METHODS AND RESULTS

Young albino rats about 25 days of age and weighing 35 to 40 grams were used. Under ether anaesthesia adrenalectomy was performed in 2 stages, i.e., one adrenal being removed 3 to 5 days after the first. The implants were hypophyses from castrated male and female rats and were placed either in the muscles, peritoneal cavity or in both. All rats received the so-called Ringer's solution for cold blooded animals as the only source of drinking water, except in 1 experiment where water was given after the third day. We have no evidence (11, 12, 13) that the precise composition of the Ringer's solution is important. The solution we used contained NaCl 0.7, KCl 0.035 and $CaCl_2$ 0.026 per cent in distilled water.

The results obtained were clear cut in that adrenalectomized rats with large ovaries were more like normal animals than were the adrenalectomized controls. This is true in respect to weight as shown in figure 1. The curve for the body weights of the adrenalectomized rats with implants approximated that of normal rats, while that for the adrenalectomized, untreated group was well below normal. It was only later, at 20 to 30 days of age, that the weights of the adrenalectomized control rats approached those of the normal group. This is due to the fact that most of the rats with severe adrenal insufficiency died within 20 days. The few surviving animals compensated in some way for the lost adrenal glands and grew more normally.

The rats with pituitary implants and large ovaries lived longer than the controls. The mean difference divided by the standard error of the difference

is significant, being 3.2 (table 1, groups H and I). This is more clearly shown in figure 2 where the death rate of 40 adrenalectomized rats analysed on the percentage basis of rats dead shows a sharp rise from the 5th to 15th day.

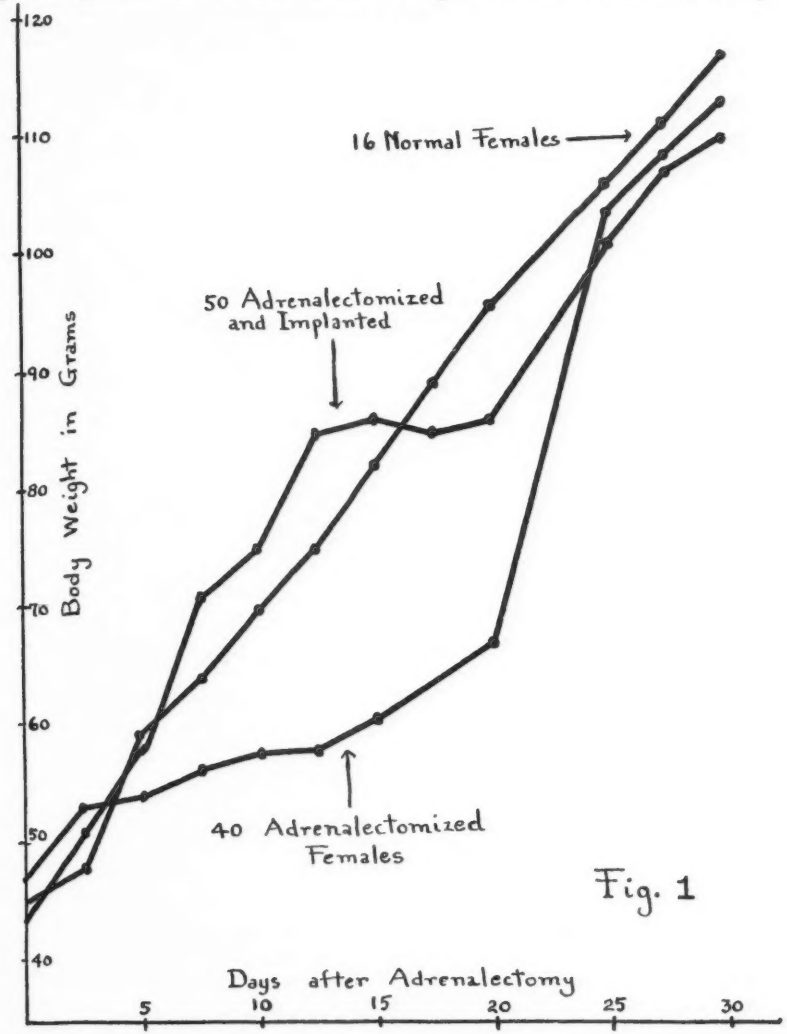
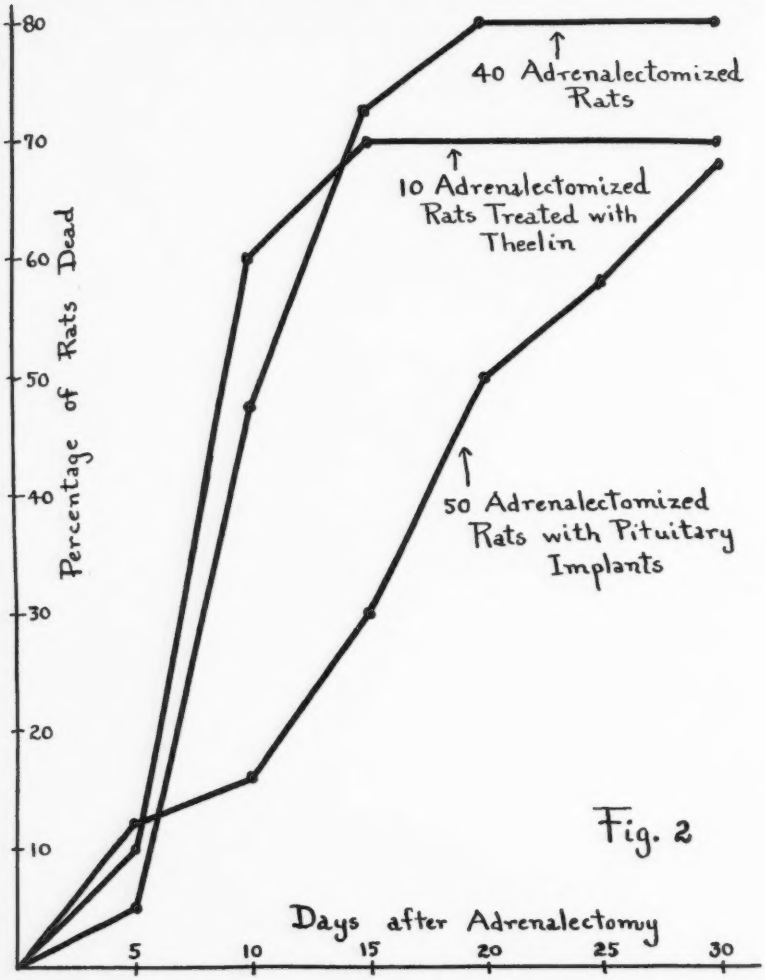


Fig. 1

A similar analysis made of 50 adrenalectomized rats with pituitary implants shows a steady death rate throughout the whole 30-day period (fig. 2). The only time the curve for the control rats is below that for the implanted group is during the first 5 days after adrenalectomy (fig. 2). This small difference is probably incidental. However, factors that may contribute to the higher death rates, during the first few days after the implants were made, are the

extra irritation caused by the implanted tissue, the higher metabolism and the large amounts of theelin given off by the ovaries. At any rate, theelin had no ameliorative effect in 10 adrenalectomized rats injected with 5 R.U. daily.



The number of cases is too small to give significant meaning to the death rate curve of the theelin injected group. The trend, however, does seem to be similar to that of the untreated controls (fig. 2).

Since theelin had no effect when injected, we were led to try other experiments to determine if the ovaries were the glands responsible for prolongation of life in these adrenalectomized rats after pituitary implants had been given. Oophorectomy was performed in 2 stages, one ovary being removed at the time each adrenal was removed, on the 25th and 30th days of age respectively.

When the ovaries were out, then the adrenalectomized rats with implants exhibited, as far as we could judge, a death rate somewhat higher than that found for adrenalectomized, uncastrated female rats (table 1, compare groups C and I). Since the pituitary implants were now ineffective in prolonging the life of these young rats (table 1, group C), it is obvious that the mechanism for the ameliorative effect was concerned with the ovaries. If enlarged fragments of the adrenal glands or accessory bodies of adrenal tissue had been present, as might have been expected following pituitary implants, the experi-

TABLE 1.

Group	Castrated	4 pituitary implants given	No. of rats and sex	Analysis of rats that lived 1 to 45 days after second adrenal removed				No. that lived more than 45 days
				Median	Mean and σ_m	Groups compared	Mean difference / S.E. of difference	
A	No	Yes	12♀	19.0	20.5±2.39	A, B	1.8	1
B	No	No	12♀	11.0	13.3±3.60	—	—	1
C	Yes	Yes	10♀	5.5	6.9±1.63	C, H	5.0	2
D	No	Yes	16♂	7.0	6.8±0.86	—	—	1
E	No	No	15♂	7.5	9.2±1.08	E, D	1.8	3
F	No	No*	10♀	10.0	12.9±1.89	F, I	0.4	None
G	No	No**	20♀	30.0	26.2±3.07	G, I	4.3	4
H	No	Yes	50♀	16.5	17.6±1.38	H, I	3.2	8
I	No	No	40♀	10.0	12.0±1.13	C, I	2.6	5

σ_m = the standard error of the mean = $\frac{\sigma}{\sqrt{n}}$. S. E. of difference = $\sqrt{(\sigma_{m_1})^2 + (\sigma_{m_2})^2}$.

* Antuitrin-S, 20 R.U. daily.
** One castrated rat hypophysis at 5 day intervals.

ments did not reveal their presence. At least in human subjects, favorable results following treatment with pituitary extract have been reported in Addison's disease by Wilder (14) and in myasthenia gravis by Simon (15). In these cases the remaining adrenal tissue was apparently stimulated to greater activity by pituitary extract.

The susceptibility of adrenalectomized animals to histamine (16) and to many other substances that put an extra burden on the animal is well known (17). We have tested the sudden withdrawal of Ringer's solution in 24 rats. The results show that the death rate of the implanted group was affected very little. The means of these groups were 20.5 and 13.3 respectively (table 1, groups A and B). It is therefore evident that the pituitary implants, through the ovaries, bring about favorable changes not only in weight and death rate but also susceptibility to extra strain as brought about by the sudden withdrawal of Ringer's solution.

In male rats pituitary implants did not have any noticeable effect on the length of life. A comparison of adrenalectomized males with and without implants may be made from the data shown in table 1 (groups D and E). The 2 groups are similar, the length of life of the individuals being slightly longer in the non-grafted rats (group E). The difference, however, between

the 2 groups is small, and it would be misleading to put emphasis upon it. It is, however, evident that the testes, unlike the ovaries, can not prolong the life of adrenalectomized rats, even when stimulated to extra activity by pituitary implants.

DISCUSSION

As the data began to accumulate it became evident that the strength of the stimulus to the ovary was important. Ordinary weak stimuli, as produced by 20 R.U. of Antuitrin-S[1] daily, had little ameliorative effect on the symptoms seen in adrenalectomized rats. The extract was started 4 days before the second adrenal was removed; this gave active corpora lutea in the process of formation, yet it did not materially prolong the life of the rats (table 1, group F). We also tried daily injections of anterior pituitary sex hormone (Antuitrin[1]), and found the rats did not live much longer than the controls. On the other hand, pituitary glands of castrated rats not only brought about the greatest increase in ovarian weights in the recipients but also prolonged the life of adrenalectomized rats to a greater extent than did either Antuitrin-S or Antuitrin. We have assumed that this difference was a matter of quantity rather than quality.

The beneficial effect of implants on the death rate reaches a maximum about the 15th day after adrenalectomy (fig. 1). Following this, the curves representing the percentage of rats dead in the adrenalectomized control and adrenalectomized implanted groups begin to approach each other. In other words, the beneficial effect of the implants begins to wane rapidly after the first 2 or 3 weeks, showing that the ovaries or secretions from the large corpora lutea are no longer able to substitute in a large measure for the lost hormones from the adrenal glands. This is interesting because the weights of the ovaries also start declining at the end of about 2 weeks after implants of 4 castrated pituitary glands are made. This we had previously pointed out (18). We also showed that when lesser stimuli, such as 1 normal male rat pituitary gland, or castrated rat serum, or pregnant human urine were given, the ovaries, after about a week, ceased increasing and began to decrease in weight. The secretion of the corpora lutea was also of only a few weeks' duration even after strong stimulation to the ovaries, as shown by the refractory condition to theelin (19).

With these facts in mind we attempted to prolong the period in which the ovaries substituted for the lost adrenal glands. One castrated rat pituitary gland was implanted in totally adrenalectomized rats every 5 days, thus insuring new stimuli and a summation effect on ovarian weight and corpora lutea activity. As a group, these rats withstood adrenalectomy better than any others. Only 50 per cent of the individuals were dead 30 days after adrenalectomy (table 1, group G). There was a significant difference between these rats and the normal adrenalectomized females. A comparison to group I shows the mean difference to be more than 4 times the standard error of the difference (table 1). This group was remarkable in that they not only withstood adrenalectomy but also an anaesthetic every 5 days.

[1] Kindly supplied by Dr. Oliver Kamm, of Parke, Davis & Co.

If one cares to say that the secretion of the corpora lutea is the active agent in these experiments, the fact should also be emphasized that old corpora lutea can not take the place of growing corpora lutea in relieving the symptoms of adrenal insufficiency. In all the implanted rats autopsy revealed large tissue masses of corpora lutea in the ovaries. The ovaries were also heavy, weighing 50 to over 150 mg., yet the rats died. In group G, table 1, the ovaries averaged 268 mg., which enhances the idea that new but not old corpora can, to a reasonable degree, substitute for the adrenal glands.

We are also cognizant of the fact that the secretion from the corpora lutea is probably not the only factor involved in these experiments. At least the improvement following injections of pituitary extract in men and male animals (14, 20) suffering from adrenal insufficiency must be explained in another way. It seems probable that other endocrine glands may be directly or indirectly involved.

SUMMARY

In a study of 200 rats adrenalectomized at 30 days of age it has been shown by several different procedures that the pituitary gland through the ovaries can ameliorate the symptoms of adrenalectomy for a period of at least 3 weeks. The stimuli to the ovaries must be strong. Thus, implants of castrated rat pituitary glands were quite effective as compared to extracts of the pituitary gland or pregnancy urine. Neither the hypophysis per se, the accessory female organs, nor the testes are significantly concerned in these beneficial effects. The effect is not due to theelin but probably is concerned with the secretion from active corpora lutea.

REFERENCES

1. Stewart, G. N., and J. M. Rogoff: Proc. Soc. Exper. Biol. & Med. **22:** 394. 1924.
2. Rogoff, J. M., and G. N. Stewart: Am. J. Physiol. **79:** 508. 1927.
3. Corey, E. L.: Physiol. Zool. **1:** 147. 1928.
4. Stewart, G. N.: Arch. Int. Med. **43:** 733. 1929.
5. Smith, P. E., and E. T. Engle: Am. J. Anat. **40:** 159. 1927.
6. Brouha, L., H. Hinglais and H. Simonnet: Bull. Acad. de Méd., Paris, **103:** 150. 1930.
7. Loeb, L., and R. B. Bassett: Proc. Soc. Exper. Biol. & Med. **26:** 860. 1929.
8. Hoffmann, Fr., and K. J. Anselmino: Klin. Wchnschr. **13:** 44. 1934.
9. Emery, F. E., and W. J. Atwell: Anat. Rec. **58:** 17. 1933.
10. Houssay, B. A., and A. Biasotti: Pflüger's Arch. f. d. ges. Physiol. **227:** 664. 1931.
11. Gaunt, R., C. E. Tobin and J. H. Gaunt: Am. J. Physiol. **111:** 321. 1935.
12. Kutz, R. L., T. McKeown and H. Selye: Proc. Soc. Exper. Biol. & Med. **32:** 331. 1934.
13. Emery, F. E., and E. L. Schwabe: Proc. Soc. Exper. Biol & Med. **32:** 910. 1935.
14. Wilder, R. M.: Proc. Staff Meet., Mayo Clinic **9:** 689. 1934.
15. Simon, H. E.: J.A.M.A. **104:** 2065. 1935.
16. Perla, D., and J. Marmorston-Gottesman: Proc. Soc. Exper. Biol. & Med. **28:** 650. 1931.
17. Britton, S. W.: Physiol. Revs. **10:** 617. 1930.
18. Emery, F. E.: Endocrinology **17:** 64. 1933.
19. Emery, F. E.: Anat. Rec. **57:** 315. 1933.
20. Swingle, W. W., W. M. Parkins, A. R. Taylor and J. A. Morrell: Proc. Soc. Exper. Biol. & Med. **34:** 94. 1936.

THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE AND SALTS UPON THE SURVIVAL PERIOD OF ADRENALECTOMIZED RATS

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A series of experiments (1) previously conducted in this laboratory upon adrenalectomized white rats provided evidence which indicated that even moderate differences in environmental temperature influence the survival period of such animals. This possibility led us to conduct the temperature studies on adrenalectomized rats presented in this paper. Because of the fact that ingestion of salts has such a marked effect on survival time of adrenalectomized animals (2, 3, 4, 5, 6, 7) variations in the salt intake of the experimental animals were included in the temperature studies.

The survival period in white rats following adrenalectomy has been variously reported. Summaries of such reports may be found in recent articles (8, 9). Some investigators have experienced high mortality and short survival periods whereas others have reported the exact opposite. In comparing the results of various investigators a number of factors which may influence survival time must be taken into consideration. Some of those of possible importance are: time and nature of the anaesthesia employed (8, 10); freedom from disease (11, 12); trauma produced at operation (10, 13, 14); value and constancy of environmental temperature (8, 9, 15, 16, 17, 18); diet before and after operation (2, 3, 4, 5, 9); strain and colony of rat used (19, 20); age (8, 9, 20); sex (21); pregnancy (8, 22). This multiplicity of factors which may influence survival time affords a number of possible explanations for the great diversity of results reported.

Influence of environmental temperature upon survival period. Hartman, Brownell and Crosby (16) found that adrenalectomized rats were susceptible to the low temperatures of 4° to 18°C. as manifested by a rapid fall in body temperature when placed under these conditions. Results of low temperature treatment of adrenalectomized rats were reported by Wyman and Tum Suden (17) who noted the slow thermic recovery of such animals when again placed at room temperature. In addition to actual temperature values some investigators consider that fluctuations of temperature exert an important influence upon the adrenalectomized animal (9, 15, 18, 19).

Influence of salt intake upon survival period. The addition of liberal quantities of salts to the diet has been demonstrated to be of importance in the prolonging of the lives of adrenalectomized animals. Rubin and Krick (2) provided a drinking solution containing the chlorides of Ca, Mg, Na and K which served to keep adrenalectomized rats alive and in apparent good health for 4 months and over. By employing the same solution Kutz, McKeown and Selye (3) and Gaunt, Tobin and Gaunt (4, 5) succeeded in prolonging the lives of adrenalectomized rats.

EXPERIMENTAL METHODS

General considerations. Previous to being placed on experiment all rats were maintained on an adequate stock diet. In order to facilitate a better regulation of salt intake, all rats on experiment were provided with a diet containing a minimum of salts, which, however, were sufficiently ample to maintain control animals and promote their growth over long periods of time. Its composition was: casein 18, crisco 8, cod liver oil 2, starch 64, brewer's yeast 8 per cent.

Because of the susceptibility of adrenalectomized animals to bacterial infections (11, 12) the health and general condition of the rats were carefully watched. Experimental animals were examined at autopsy for both gross and histological evidence of infection or any pathological condition not related to adrenal insufficiency; in a few instances lung infections were observed and such animals were not included in our averages. In order to insure a better comparison litter-mates were as evenly distributed as possible in all of the experimental groups. In a further effort to obtain offspring more closely approaching each other in hereditary similarity a single male animal was permitted to sire a majority of the rats used in the experiments.

Operative procedure. The hair was removed from the operative site by plucking. The animals were then subjected to light ether anaesthesia which was usually not effective for more than 10 to 12 minutes since the operation seldom required more than 6 or 7 minutes to perform. Bilateral adrenalectomy was accomplished through 2 lumbar incisions. When sufficiently exposed the fascia and root structures of the gland were firmly grasped with a fine forceps and dissected away together with the gland. Special care was taken to remove as much of the surrounding fat and fascia as possible. This was accomplished with a minimum of bleeding. The wounds healed readily and in no instance was there evidence of infection.

Post operative care. The temperatures of 37°, 35°, 10° and 4°C. were accurately maintained by thermostatic control. Abundant circulation of air was provided at all times. Most of the work was performed during the winter months when the temperatures of the 22° and 30° rooms did not vary appreciably. The 22° room never varied more than 2 degrees either way and the 30° room, except in rare instances and but for short periods only, reached as low as 27.5° and as high as 31.5°. The animals were placed on experiment the day following adrenalectomy. They were so divided that at each temperature 1 group was maintained on the drinking solution used by Rubin and Krick (2) and the other group on distilled water. These groups were kept at the various temperatures of 4°, 10°, 22°, 30°, 35° and 37°C. Individual distribution was effected so that litter mates were represented among groups at the various temperatures and drinking solutions.

RESULTS

A total of 284 rats were used in the experiments. Of the 114 adrenalectomized rats placed on high salt intake, 18 animals, by continued survival for much longer periods than their litter mates, gave promise of living on indefinitely. As a criterion of insufficiency such animals were given distilled water in place of the salt solution with the result that death rapidly followed in many. Such animals were considered markedly deficient and are included in the tables. Eight which lived on in apparent good health, even after being

placed on distilled water, were killed. Histological sections of the fascia about the site of operation revealed accessory cortical tissue in all excepting 1. One animal on high salt intake which died of apparently uncomplicated adrenal insufficiency showed accessory cortical tissue upon histological examination. These animals are not included in the tables.

Of the 127 adrenalectomized rats on low salt intake, 2 animals which died with apparently uncomplicated adrenal insufficiency showed histological evidence of accessory cortical tissue. One animal of this group which gave

TABLE 1. SUMMARY OF RESULTS SHOWING THE EFFECT OF ENVIRONMENTAL TEMPERATURE UPON THE DURATION OF LIFE OF ADRENALECTOMIZED RATS.

Temp. °C.	With Distilled Water for Drinking					With R-K. Salt Solution for Drinking				
	No. of Rats	Range Days	Duration of Life		Av. Deviation (median)	No. of Rats	Range Days	Duration of Life		Av. Deviation (median)
			Average	Median				Average	Median	
4	8	1–5	2.6	2.5	1.6	7	1–2	1.1	1	0.3
10	9	1–10	5.5	4	2.7	10	1–11	4.7	5.5	2.0
22	68	4–13	7.8	7	2.1	35	8–91	44.5	36	22.5
30	11	5–15	6.7	6	2.1	16	8–113	57.0	66	31.5
35	20	3–13	6.7	6	1.8	18	4–48	20.4	12.5	10
37	8	3–6	3.9	3.5	0.9	19	3–47	15.4	9	8.2

promise of living on indefinitely was killed and upon examination revealed accessory cortical tissue. These animals are not included in the tables.

The mortality of the group on high salt intake was 93 per cent whereas in the group on low salt intake it was 99.2 per cent. Five of the animals in which evidence of pulmonary infection was noted are not included in the tables or mortality averages.

The 17 unoperated control animals on high salt intake which were kept at the various temperatures thrived and gained in weight. Of the 21 unoperated control animals maintained on a low salt intake, at the various temperatures all remained in apparent good health and gained in weight with the exception of the animals kept at 37°. All of the 9 young control animals kept at 37° on a low salt intake had an average survival period of 13.8 days with a median of 14 days.

Experimental results from a series of 229 adrenalectomized young rats are shown in table 1. The rats maintained on the low salt diet supplemented by distilled water for drinking died uniformly. The average and median survival periods were fairly constant for the temperatures of 22°, 30° and 35°, with decreased survival periods at lower and at higher temperatures. When the low salt diet was supplemented with the salt solution for drinking, the survival period was greatly but not indefinitely lengthened, the rats finally dying with the symptoms of adrenal insufficiency. In this group there was a greater variation of the survival period with temperatures than among those on water. The length of life was much greater at 22° and 30° than at higher or lower temperatures, with a maximum or apparent optimum at 30° among the temperatures employed.

The animals used in the experiments were young rats most of which were less than 75 grams in weight. By separating the rats into 2 age or weight groups, those less than 50 grams and those between 51 and 75 grams, the results shown in table 2 are obtained. As the animals were young, of uniform stock and on an adequate diet prior to adrenalectomy, separating them according to either age or weight has the same significance.

The increase in length of survival period with age among the groups given water to drink is similar to that reported by others (8, 21), although the values

TABLE 2. RESULTS SHOWING THE EFFECT OF AGE AND ENVIRONMENTAL TEMPERATURE UPON THE THE DURATION OF LIFE OF ADRENALECTOMIZED RATS WITH DISTILLED WATER FOR DRINKING.

Temp. °C.	Body Weight Less than 50 grams					Body Weight 51 to 75 grams				
	No. of Rats	Range Days	Duration of Life		Av. Deviation (median)	No. of Rats	Range Days	Duration of Life		Av. Deviation (median)
			Average	Median				Average	Median	
4	—	—	—	—	—	2	1	1	1	0
10	5	1–4	3	3	1	—	—	—	—	—
22	17	5–10	6.7	6	1.4	43	5–12	8.1	8	2.1
30	4	4–6	5	5	0.4	7	5–15	8.4	7	2.3
35	13	3–10	5.8	6	1.3	2	3–13	8.0	8	5
37	—	—	—	—	—	2	4	4	4	0
	WITH R-K. SALT SOLUTION FOR DRINKING									
4	—	—	—	—	—	2	1	1	1	0
10	4	1–3	1.8	1.5	1	6	5–11	7.7	7.5	1.8
22	19	8–91	42.1	29	25	16	12–89	48.1	52	23
30	10	8–112	46.5	40	27	6	33–113	74.5	83.5	29.8
35	7	7–45	16	11	4	11	4–48	22.2	24	11
37	10	1–12	7.3	9	2.9	7	3–47	21	11	16

are slightly higher. Similarly, when given salt solution to drink the values are higher in the older group. The animals of both age groups on a high salt intake show an apparent maximum in duration of life at the temperature of 30.°

SUMMARY

The lives of adrenalectomized rats with severe adrenal insufficiency were markedly although not indefinitely lengthened by the salt solution of Rubin and Krick. The use of this salt solution serves to diminish the mortality of adrenalectomized rats. The survival period with a high salt intake was shortened by either high or low environmental temperatures with an apparent optimum for maximum survival at 30°C. Young adrenalectomized rats of from 37 to 50 grams' weight showed a shorter survival period than did older rats ranging from 51 to 75 grams' weight. A total of 284 rats was used in the experiments.

REFERENCES

1. Norris, E. R. and R. S. Weiser: Unpublished work.
2. Rubin, M. I. and E. Krick: Proc. Soc. Exper. Biol. & Med. **31:** 228. 1933.
3. Kutz, R. L., T. McKeown and H. Selye: Proc. Soc. Exper. Biol. & Med. **32:** 331. 1934.
4. Gaunt, R., C. E. Tobin and J. H. Gaunt: Proc. Soc. Exper. Biol. & Med. **32:** 134. 1934.

5. Gaunt, R., C. E. Tobin and J. H. Gaunt: Am. J. Physiol. **111:** 321. 1935.
6. Swingle, W. W., J. J. Pfiffner, H. M. Vars and W. M. Parkins: Am. J. Physiol. **108:** 159. 1934.
7. Loeb, R. F., D. W. Atchley and J. Stahl: J.A.M.A. **104:** 2149. 1935.
8. Firor, W. M. and A. Grollman: Am. J. Physiol. **103**: 686. 1933.
9. Agate, F. J. and R. L. Zwemer: Am. J. Physiol. **111:** 1. 1935.
10. Stewart, G. N.: Psysiol. Rev. **4:** 163. 1924.
11. Jaffe, H. L.: Am. J. Physiol. **78:** 453. 1926.
12. Steinbach, M. M.: Proc. Soc. Exper. Biol. & Med. **27:** 142. 1929.
13. Freed, S. C.: Proc. Soc. Exper. Biol. & Med. **30:** 677. 1933.
14. Donahue, J. K. and W. M. Parkins: Proc. Soc. Exper. Biol. & Med. **32:** 1249. 1935.
15. Scott, W. J. M.: J. Exper. Med. **38:** 543. 1923.
16. Hartman, F. A., K. A. Brownell and A. A. Crosby: Am. J. Physiol. **98:** 674. 1931.
17. Wyman, L. C. and C. Tum Suden: Am. J. Physiol **89:** 362. 1929.
18. Belding, D. L. and L. C. Wyman: Am. J. Physiol. **78:** 50. 1926.
19. Gaunt, R.: Am. J. Physiol. **103:** 494. 1933.
20. Gaunt, R., J. H. Gaunt and C. E. Tobin: Proc. Soc. Exper. Biol. & Med. **32:** 888. 1935.
21. Sisson, E. D. and B. March: Endocrinology **19:** 389. 1935.
22. Stewart, H. A.: Proc. Internat'l. Congress Med. London, Sec. 3. P. 173. 1913.

CLINICAL AND LABORATORY NOTES

AN INVESTIGATION OF THE PRESENCE OF ESTROGENIC HORMONES IN THE OVA AND OVARIES OF FISH*

ABNER I. WEISMAN, CHRISTOPHER W. COATES AND ROSALIND L. MOSES

In the past few years cancer and endocrine research and many other types of investigations with fish have been reported.[1, 2, 3, 4, 5] The female bitterling, Rhodeus amarus, has been the object of a great deal of experimentation with regard to the sex hormones and it has been suggested that the elongation of its ovipositer is produced by the estrogenic hormones.[6, 7] Experiments have been performed by injecting directly into the fish and by merely adding the substances to the water containing the fish, to prove that the estrogenic substances are ovipositor-lengthening. A test for pregnancy using these fish has been suggested.[8] Very little is known regarding the normal hormone control of the ovipositor-elongation phenomenon which occurs regularly in the breeding season of the bitterling. Moreover, there are no data available as to whether the gonads of fish contain hormones that are estrogenic in nature or otherwise.

For this reason it was decided to investigate the ova and ovaries of fish to determine, if possible, whether or not hormones could be extracted from the gonads as in the mammals. It was not considered feasible to use the bitterling for extraction purposes since the fish has to be imported from Europe, and since the size of the fish ranges from 1 to 1.5 inches and weighs but a small fraction of an ounce. Moreover, if ovulation in this fish is hormone-controlled as it is in mammals, birds and amphibia it is a fair assumption that ovulation in other teleosts is similarly controlled. Consequently, for the initial investigation much larger teleosts were used. These were the common whitefish, Coregonus clupeiformis, a fresh water form, and the common herring, Clupes harengus, a marine form.

Experimental. A. The ova of 2 common white fish, just prior to the spawning season of the fish, when there were thousands of almost ripe ova present, were ground with sand. They were then extracted using a method described by Harrow and Sherwin[9] (employed in routine urine extraction for estrogenic hormones) involving acidification, chloroform extraction, saponification, refluxing and re-extraction with ether and chloroform. The final product consisted of 2 substances, one oily and the other of a gummy consistency. Both fractions were made viscous enough for injection into immature mice by mixing them separately with olive oil and then

* From the Department of Medicine of the French Hospital, and from the New York Aquarium, New York.

[1] Tozawa, T.: Folia. anat. japon. **7**: 407. 1929.
[2] Wunder, W.: Ztschr. f. Vergleich. Physiol. **13**: 696. 1931.
[3] Zondek, B., and H. Krohn: Klin. Wchnschr. **11**: 405. 1932.
[4] Saphir, W.: Proc. Soc. Exper. Biol. and Med. **31**: 864. 1934.
[5] Szusz, F.: Orvosi hetil. **77**: 905. 1933.
[6] Fleischmann, W., and S. Kann, Pflüger's Arch. f. die ges. Physiol. **230**. 662. 1932.
[7] Kanter, A. E., C. P. Bauer, and A. H. Klawans: J.A.M.A. **103**: 2026. 1934.
[8] Owen, S. E.: Endocrinology **20**: 214. 1936.
[9] Harrow, B., and C. P. Sherwin: The Chemistry of the Hormones. Williams and Wilkins, Baltimore, 1934.

injected according to the Ascheim-Zondek technique into separate groups of infantile mice. After 96 hours there were no signs of estrus, uterine growth or tubal swelling. The vaginal smears were entirely negative. The entire procedure was then repeated using a press to crush the ova instead of grinding with sand, with the same end result.

B. The ovaries of 30 common herrings in the resting phase of the ovulatory cycle, were carefully removed from the fish and crushed under a press. The extraction was continued as in A with 3 fractions resulting as the end products. One was of a thin oily consistency, the second was a thicker oil, and the third was gummy in nature. They were individually mixed with olive oil and injected into immature female mice as above. After 96 hours there were no signs of estrus.

Comment. Ova of whitefish contain no estrogenic hormone or too small an amount to produce estrus in the immature mouse. Herring ovaries likewise failed to give positive estrus reactions when crude extracts were injected into immature mice. One cannot draw any general conclusions from the above experiments except to say that if fish do secrete hormones they are probably not of the estrogenic type. Although many investigators have shown that the ovipositor elongation in the bitterling may be due to the estrogenic hormone, we are not certain that there is a spontaneous production of estrogenic hormones in fishes at all.†

Conclusions. Extracts of the ova of whitefish and the ovaries of the common herring were tested for the presence of the estrogenic hormones. The ova and ovaries either do not contain the estrogenic hormones or the hormones are not present in large enough amounts to produce the estrus reaction in mice.

† Since the writing of this paper, Kleiner has reported positive ovipositor tests with male urines and fractions containing male hormones, and has since published in the J.A.M.A. a new test entitled The "Female Bitterling as a Test for Male Hormone."

THE ISOLATION FROM OVARIAN TISSUE OF A CRYSTALLINE SUBSTANCE POSSESSING HIGH ESTROGENIC PROPERTIES

ROBERT H. ANDREW AND FREDERIC FENGER[1]

For a number of years we have observed crystalline formations after the removal of cholesterol in ovarian concentrates obtained by direct extraction and fractionation of fresh and desiccated ovaries. While the highly purified concentrates were potent, we did not succeed in separating crystals in sufficiently pure form to ascertain their full estrogenic properties.

In the course of a series of studies carried out on the nature of unsaponifiable substances of various tissues, we have been able to isolate from ovaries a crystalline substance possessing chemical and physiological properties quite different from those characteristic of the urinary estrus-producing substances.

For practical reasons we employed the entire gland as the basic material for the work presented here. The correct anatomical separation of the graafian follicles, the corpora lutea, the colored scar tissue left after atrophy of the corpora lutea, and the remaining so-called ovarian residue from hundreds of pounds of ovaries is a tedious and expensive procedure. Another consideration is that the graafian follicles, the corpora lutea and the ovarian residue as separated by hand all possess some estrogenic properties. While little is known concerning the exact interrelation of the various structures of the gland, we do know that follicles are produced and corpora lutea are formed and disappear during the entire normal reproductive period. Confronted with so many unknown factors, it was decided to follow what appeared to be the most direct procedure first and then work out the details later.

Early in our investigation we were impressed by the difference in behavior of spayed rats towards estrogenic substances derived from metabolic waste products, such as theelin and theelol, and the true ovarian hormone or hormones. Rats injected with the proper amounts of theelin or theelol usually come into estrus in from 48 to 72 hours and remain in heat only a short time. When the purified concentrates or crystals as prepared by us from whole ovaries are administered, it requires approximately 96 hours to produce estrus regardless of the dose given, but the animals remain in heat for a considerable length of time; in the case of a relatively large dose, for several days. The behavior of the animals during this prolonged estrus is characteristic; there is no doubt about their being in heat.

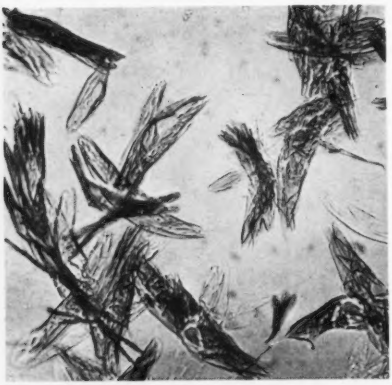
For the isolation of the crystalline fraction we employ the following procedure. The fatty portion of whole ovaries is saponified rapidly with alcoholic potash at low temperature. The resulting soap solution is diluted with water and the unsaponifiable material extracted in the customary way. After removal of alkali and evaporation of the fat solvent, the unsaponifiable material appears as beautiful golden-yellow glistening crystalline masses containing a high percentage of cholesterol. It may be mentioned here that the amount of fatty substances extractable from ovaries as well as the unsaponifiable fraction therefrom varies considerably. In one instance we obtained 15 lb. of fatty material from 460 lb. of hog ovaries. These 15 lb. yielded 505 grams of unsaponifiable material. In another instance 30 lb. of fatty

[1] From the Research Laboratory in Organotherapeutics of Armour and Company, Chicago, Illinois.

substance furnished only 728 grams of unsaponifiable residue. This was probably due to interstitial and adherent fat.

The unsaponifiable material is boiled with 3 volumes of alcohol and the alcohol thoroughly chilled to facilitate the precipitation of cholesterol. The filtrate is mixed with dilute aqueous caustic soda. The resulting precipitate is removed and the alkaline liquid evaporated. The oily residue is mixed again with dilute aqueous caustic soda, heated and filtered. The clear brownish-colored liquid is extracted with ethyl ether. After separation the aqueous layer is run off and the clear ether extract is evaporated to dryness. The straw colored residue is taken up in a small volume of absolute alcohol by aid of gentle heat, filtered, and the filtrate is set aside at 5°C. for crystallization. The substance is recrystallized from absolute alcohol. It is now too early to speak about yields. Under the microscope the crystals appear as colorless clusters of leaflets (see photograph).

We have made only a few batches of these crystals. Preliminary work—which needs confirmation—gives the following data.



Melting point (uncorrected)	95.1°C.
Molecular weight (Rast)	320
C (Pregl)	73.47%
H (Pregl)	12.32%
N (Pregl-Dumas)	4.43%
O (by difference)	9.78%

This analysis indicates the probable emperical formula as $C_{20}H_{41}O_2N$.

The low melting point and the fact that it contains nitrogen distinguishes our substance from the heretofore reported natural estrogenics. Biological assays on spayed rats indicate that 0.00001 mg. of the substance dissolved in oil produces positive estrus in spayed rats after 96 hours. The animals remain in estrus for at least 24 hours with a gradual return to the resting stage.

Physiologically, this product differs from the estrogenic substances derived from urine in that a somewhat delayed but more persistent estrus is produced. Due to the limitations of the present bioassay method and the apparent physiological differences between the urinary hormones and this, we are not presenting in this report a comparison of potency (in rat units) between our crystals and other estrogenic substances. Present indications, however, are that the crystals possess estrogenic properties far in excess of theelin and theelol. In carrying out the assay work, control tests with standard theelin were made in order to ascertain that the groups of rats employed were suitable and reliable for assay purposes.

Further work is in progress and will be reported at a later date.

Summary. We have been able to isolate from ovaries a crystalline substance possessing a melting point of 95.1°C. (uncorrected), a molecular weight of 320 (Rast) and of the following composition: carbon 73.47, hydrogen 12.32, nitrogen 4.43 and oxygen 9.78 per cent.

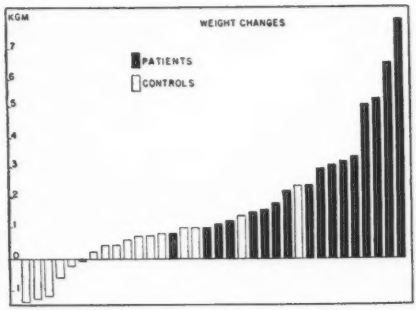
The potency of the isolated substance appears to be such that 0.00001 mg., when injected in one dose into spayed rats, will produce positive estrus after 96 hours. The animals remain in heat for at least 24 hours before a gradual return to the resting stage occurs. The crystalline ovarian substance differs both chemically and physiologically from the estrus-producing products derived from urine.

WEIGHT CHANGES FOLLOWING THE USE OF GLYCERIN EXTRACT OF ADRENAL CORTEX[1]

R. G. HOSKINS AND H. FREEMAN

In previous publications[2] attention was called to the gain in weight following the use of a commercial preparation of adrenal cortex.[3] Recently the study has been supplemented by an additional series of cases in which the preparation was given in standard dosage of 1 gm. (fresh gland equivalent) 3 times daily to 17 schizophrenic patients and 17 normal men. The treatment was continued for a period of 3 weeks.

In general the results added nothing significant to the data previously reported except that the difference in pressor response between patients and controls was less than in the previous series and differences in blood cholesterol were not evident but the weight changes came out more sharply.



Graph showing changes in weight undergone by 17 schizophrenic patients and 17 normal men treated for 3 weeks with glycerin extract of adrenal cortex by mouth.

The current series is rather more satisfactory than those previously reported in that the number of subjects is greater and the patients and controls were treated as pairs each individual of which was exposed to the same environmental conditions as his mate. Both lived in the same ward, received the same food, were studied by the same personnel and underwent the same tests. Thus seasonal as well as other possibly significant factors were rather well controlled.

The accompanying chart shows graphically the essentially features of the study as regards weight. It is seen that 1 control subject lost 1.4 kg. during the period of medication while, at the other extreme, 1 patient gained 7.8 kg. The other weight changes fell between these limits. All the patients showed a gain, the range being from 0.8 to 7.8 kg. and the average gain being 3.0 kg. The range in the normal subjects was from —1.4 to +2.4 kg. with an average gain of 0.3 kg. This latter value is not statistically significant. There was little overlapping in the distributions of the two series. The difference in mean weight gain between patients and controls is, statistically, highly significant. It is the more noteworthy in that schizophrenic subjects are more resistant than normal to general measures designed to increase their weight.

We have not been able to carry out controlled metabolic studies to determine how the weight increase was brought about but since all the subjects were on ad libitum diets it is probable that the explanation lies in increased appetite. At any rate,

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[1] From the Memorial Foundation for Neuro-Endocrine Research and the Research Service of the Worcester State Hospital, Worcester, Massachusetts.

[2] Hoskins, R. G. and H. Freeman: Endocrinology **17**: 29; 677. 1933.

[3] We are further indebted to Schieffelin & Co. of New York for generous supplies of the extract used.

Rogoff has stated that patients suffering from adrenal deficiency develop an aversion to fatty food and that treatment with adrenal cortical extract seems to restore the appetite.

Summary. Glycerin extract of adrenal cortex was administered by mouth for 3 weeks to 17 schizophrenic patients and 17 normal men living in the same environment. There was no significant change in weight in the controls, but the patients without exception showed a gain, the average being 3.0 kg.

A CASE OF ADRENAL CORTICAL TUMOR IN AN ADULT MALE CAUSING GYNECOMASTIA AND LACTATION

H. LISSER

Adrenal cortical tumors in the adult male producing endocrine disturbances are rare. The privilege of recording another case, not my own, is due to courteous permission granted by the Commanding Officer of the Letterman General Hospital, Presidio, San Francisco.*

Inasmuch as cortical hyperfunction masculinizes the little girl and the adult female and causes a young boy to develop the genitalia and sexual characteristics of an adult male, it might be supposed that the same sort of tumor or hyperplasia, when it occurs in a man, would intensify masculine traits, but curiously enough such does not seem to be the case. Indeed, the very opposite takes place; the man becomes feminized. This peculiar phenomenon would appear to be paradoxical.

A remarkable case published by Holl[1] in 1930 illustrates this. A man aged 44 years, the father of two healthy sons, had been a gymnastic instructor, virile and athletic. During the previous 2 years his breasts enlarged in characteristic feminine fashion; they became tender and mammary glands similar to those of a woman could be felt; marked pigmentation of the nipple areolae developed, and a congested network of veins became apparent around the nipples and over the breasts. Masculine traits diminished or disappeared; the testicles gradually atrophied, the penis became smaller, libido vanished and intercourse ceased; erections and pollutions no longer occurred. No homosexual tendency developed; he was merely sexually indifferent. He became obese, his facial expression altered astoundingly so that friends sometimes passed him on the street and failed to recognize him; the features became weak for a man. Gradually acne appeared on the arms, legs and trunk. Hardly any hairs remained on the chest and the pubic escutcheon became rather sparse though of masculine type.

A varicocele developed on the left side; the left breast was larger than the right and the left nipple was more deeply pigmented; the lower pole of the left kidney could be felt and an x-ray film showed a fist-sized tumor shadow above the right kidney, the latter being two finger breadths lower than normal. The blood pressure was normal. A preoperative diagnosis of left adrenal tumor causing incretory disturbances was made. The tumor was removed, together with the left kidney. The tumor was well encapsulated, as is usually the case with adrenal cortical tumors, of a sulphur yellow color, with many cystic areas and composed of adrenal cortical cells resembling the fascicular zone. There was no evidence of metastases.

A week after the operation the venous engorgement of the breasts had disappeared and they were far less tender; gradually the breasts decreased in size, the Montgomery glands became smaller and pigmentation vanished. One month postoperatively, pollution occurred, erections and libido returned and later he resumed normal intercourse with his wife. The varicocele disappeared. The panniculus adiposus diminished. The penis and testicles regained their former size. The masculine

* I appreciate the kindly coöperation of certain members of the Staff, who obtained the clinical records and autopsy reports for my perusal and granted opportunity to study sections of the tumor.

[1] Holl, G.: Deutsche Ztschr. f. Chir. 226: 276. 1930.

facial expression was restored and the patient felt livelier. In other words, he was remasculinized. This extraordinary case constitutes probably the first instance of recognition and successful removal of an adrenal cortical tumor in an adult male with cure of the internal secretory disturbances. It is entirely comparable in its remasculinization, to the refeminization which occurs following the removal of an adrenal cortical tumor from an adult female.

Holl reports another case in a 15 year old boy who had gynecomastia, a striking linea nigra, an inoperable huge tumor the size of a man's head, above the right kidney. The boy died but autopsy was refused.

Bittorf,[2] reported the case of a 26 year old man with gynecomastia and testicular atrophy, loss of potency, and a left sided adrenal cortical tumor; subsequently there developed metastases to the liver, cachexia and death. The testicles contained hardly any interstitial cells but good spermatogenic tissue. The necropsy was made by Mathias who also reported the case.[3]

Zum Bush[4] and Parkes-Weber[5] reported the same case of a 27 year old man who had painless gynecomastia and lactated. At autopsy a malignant 'hypernephroma' was found on the left side, with metastases to the lungs, pleura and mesenteric glands. The mammary glands, microscopically, were typical of the fully developed secreting female breast.

These 4 cases would seem to be the only published instances of adrenal cortical tumors in adult males, causing endocrine abnormalities.

Case Report. A Veteran (R. M. B.), aged 33 years, was admitted to the Letterman General Hospital, San Francisco, June 17, 1925. He complained of pain in the left flank, which radiated at times to the left shoulder blade and also down into the left testicle. This had troubled him for 1 month. He also occasionally spat up bloody sputum. There was no history of hematuria but it was noted that urination tended to relieve the pain in the flank. His average normal weight had been 145 pounds. On admission he weighed 135 pounds.

He was markedly tender to 'kidney punch' on the left side, and a firm mass could be felt in the left upper quadrant. Both breasts were markedly enlarged and firm but they were not tender to pressure. A watery fluid could be expressed at times from the breasts. There was considerable hair growth over the chest and abdomen; this masculine trait evidently had not been affected, despite the gynecomastia. The blood pressure was 112/88. The urine findings were normal except for the presence of red cells. The phenosulphonephthalein test and a flat roentgenogram of the kidney regions gave normal findings. The Wassermann reaction was negative. A pyelogram on the left side showed the calices to be squeezed together as if something was pressing or encroaching upon them. The right side was normal. Cultures of the urine obtained at cystoscopy were sterile. A roentgenogram of the chest disclosed metastases in both lungs.

The patient grew weaker gradually and during the few weeks in the hospital ran a slight fever. He died on July 18, 1925. The clinical diagnosis was "left hypernephroma with metastases in both lungs, liver and spleen."

Autopsy. The body was that of an emaciated man who looked to be nearer 40 years of age, than 33. A mass, the size of a large fist, was found, firmly adherent to the bodies of the second, third and fourth lumbar vertebrae; it surrounded the aorta, vena cava, and coeliac axis; it was roughly lobulated, firm and of a yellowish color. The left suprarenal was closely adherent to the mass, but careful dissection showed the adrenal and the kidney seemingly to be entirely free from the tumor

[2] Bittorf, A.: Berl. klin. Wchnschr. **56**: 776. 1919.
[3] Mathias, E.: Virchows Arch. f. path. Anat. **236**: 446. 1922.
[4] zum Busch, J. P.: Deutsche med. Wchnschr. **53**: 323. 1927.
[5] Parkes Weber, F.: Lancet **1**: 1034. 1926.

and evidently not the point of origin. The mass arose from retroperitoneal tissue, probably from an adrenal 'rest.' The tumor weighed 625 grams.

Microscopic Description. Sections from the adrenal tumor submitted showed a comparatively small-celled growth arranged in a pseudoadenomatous manner. Most of the cells had a light bluish staining cytoplasm in contrast to those found in the usual malignant cortical tumor. There were some large multinucleated cells similar to those found in a comparatively benign adenoma. Other sections showed the large clear cells, with non-staining cytoplasm, which one associates with the so-called hypernephroma. These appeared only, however, in the metastases in the lungs and seemed to be a malignant variant of the primary tumor cells. The tumor was definitely of cortical origin and could be called a malignant adenoma in the sense that it probably began as a definitely encapsulated tumor producing excessive hormone but metastasizing as such tumors do if not promptly removed. Diagnosis: adrenal cortical carcinoma with metastasis to the lungs.†

Discussion. An 'indispensable-to-life-hormone' has been isolated (independently by Hartman, and by Swingle and Pfiffner) but administration of excessive amounts of this substance to animals has not produced sexual precocity convincingly. Corey and Britton[6] reported changes in the ovaries and testes of rats following such treatment, which they interpreted as a stimulation to sexual maturity, but Connor[7] was unable to confirm this. Atwell[8] has found that the ovaries of hypophysectomized tadpoles become larger in animals treated with extract containing cortin than in untreated controls. This observation indicates that the action is not through the pituitary. Hartman, Greene, Bowen and Thorn[9] have shown that the interval between menstrual periods can be shortened, 3 to 5 days in normal women by the injection of cortical extract. It does not necessarily follow that a supposed excess of cortin in an adrenal cortical tumor accounts for the amenorrhea of virilism, although there is evidence that the adrenal cortex contains a substance which inhibits the activity of the gonads of mature animals.

Perhaps most interesting of all this experimental data is the discovery by Brownell, Lockwood and Hartman[10] of a new hormone in the adrenal cortex which supports lactation and for which they have suggested the name cortilactin. One is tempted to wonder whether an excess of this cortilactin in an adrenal cortical tumor might be the explanation for the lactation reported to have occurred in the patient herein cited as well as in the case reported by zum Busch. If such a supposition were true, then why does lactation not occur in the adult woman afflicted with virilism, presumably caused by the same pathological lesion? We must leave these perplexities to the future but meanwhile clinicians can be awake to the existence of these syndromes caused by adrenal cortical tumors and hyperplasias, and make every effort to recognize them early and operate promptly.

Summary. A case of adrenal cortical tumor is reported in an adult male, which caused endocrine disturbances in the direction of feminization, namely gynecomastia and lactation. There are 4 other similar proven cases in the literature. All the patients died except one, reported by Holl, in whom removal of the tumor was followed by remasculinization and complete cure.

Fitzhugh Bldg.
San Francisco, California

† Through the courtesy of the Pathological Staff of The Letterman General Hospital I was permitted to borrow some of the sections from the tumor and metastases; these were submitted to Dr. C. L. Connor, University of California Medical School who made the above report. His coöperation is gratefully acknowledged.

[6] Corey, E. L. and S. W. Britton: Science 74: 101. 1931. Also Am. J. Physiol. 99: 33. 1931-32.
[7] Connor, C. L.: Proc. Soc. Exper. Biol. & Med. 29: 131. 1931.
[8] Atwell, W. J.: Proc. Soc. Exper. Biol. & Med. 29: 1259. 1932.
[9] Hartman, F. A., C. W. Greene, B. D. Bowen and G. W. Thorn: J.A.M.A. 99: 1478. 1932.
[10] Brownell, K. A., J. E. Lockwood and F. A. Hartman: Proc. Soc. Exper. Biol. & Med. 30: 783. 1933.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

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John C. Donaldson	Edward Larson	D. L. Thomson
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ADRENALS

Neuroblastoma of the adrenal in children. Askin, J. A. and C. F. Geschickter, J. Pediat. 7: 157. 1935.

The findings are reported in 17 cases of neuroblastoma of the adrenals in children ranging in age from birth to 13 years. The prominent symtoms were abdominal mass, pallor, fever, enlarged liver, abdominal pain and bone changes. The authors enter into a comprehensive discussion of the salient clinical features and pathological findings in tumors of the adrenal medulla.—M. B. G.

Influence of induced adrenal cortical hypertrophy and of cortical extract on resistance to certain toxins (Influence de l'hypertrophie provoquée de la cortico-surrénale et de l'extrait cortical sur la résistance à certaines intoxications). Benetato, G., I. Gaina and C. Oprisiu, Compt. rend. Soc. de biol. **120:** 353, 1935.

Adrenal hypertrophy was produced in 20 rats by means of anterior hypophyseal extract. These rats and 20 more controls were injected intraperitoneally with minimal lethal doses of typhoid vaccine or with morphine. As many deaths occurred in one group as in the other. In a third group of 20 rats previous treatment with a cortical extract (not identical with cortin) was ineffectve in reducing the mortality caused by morphine injections.—E. Cutuly.

Adrenal cortical tumors. Cahill, G. F., R. F. Loeb, R. Kurzrok, A. P. Stout and F. M. Smith, Surg., Gynec. & Obst. **62:** 287. 1936.

A detailed study of 10 cases of adrenal cortical tumors is presented showing metabolic and secondary sex changes. Five had proven adrenal cortical tumors, the second 5 had no demonstrable evidence of tumor, though the symptoms were suggestive or characteristic of the disease. Adrenal cortical tumors may be classified as (a) tumors with endocrine changes. These are (1) pseudohermaphroditism (female), (2) premature puberty in the male, (3) growth maturity with masculinity in female children, (4) hirsutism, amenorrhea, and adiposity in adult females, (5) after menopause, an indistinct picture with adiposity, (6) possible inversion changes in male adults. (b) Tumors with only an abdominal mass. (c) Tumors with metastatic masses. (d) Tumors with arterial hypertension either paroxysmal or continuous. Symptoms vary according to sex, age, and degree of malignancy. Metastatic masses produce the same endocrine changes as the normal tumor. Enlargements or tumors of the adrenal may be diagnosed by means of x-ray, either through the density of the tumor shadow or the tumor displacing the kidney, or by means of injection of air in the perirenal fat. The transperitoneal route is advocated in the surgical removal of these masses. The authors have been unable to demonstrate the adrenal cortical hormone as cortin from any of these tumors.—L. B. S.

A suggested test for functional cortical adrenal tumor. Frank, R. T., Proc. Soc. Exper. Biol. & Med. **31:** 1204. 1934.

In a case of 'basophilic adenoma' repeated hormonal studies were made. At autopsy the pituitary was found on serial section to be normal. A large carcinoma of the adrenal cortex was found. A second case showed at autopsy a large carcinoma of the adrenal cortex with metastasis to the liver. Extracts from the tumor showed twice as much female sex hormone as did control tissues. Excretion of female sex hormone in the urine was at times increased to a degree seen normally only in pregnancy but there was no increase in pre-pituitary and female sex hormones circulating in the blood.—R. C. Moehlig.

Influence of gestation on the survival of adrenalectomized animals (Influence de la gestation sur la durée de survie chez les animaux surrénalectomisés). Gradinesco, A., N. Santa and F. Lucan-Jonesco, Compt. rend. Soc. de biol. **120:** 356. 1935.

The survival period following bilateral adrenalectomy was shorter in pregnant dogs and cats than in non-pregnant animals. Since other experimenters seem to have shown that the adrenal cortical hormone may pass the placenta, the present study indicated that the fetal supply of this hormone is not endogenous but maternal.—E. Cutuly.

The role of the hypophysis in experimental chronic adrenal insufficiency. Grollman, A., and W. M. Firor, Am. J. Physiol. **112:** 310. 1935.

A state of chronic partial adrenal insufficiency was induced in rats, cats and dogs by various methods. The cessation of growth, failure of normal reproductive activity, and subnormal body temperature which ultimately manifested themselves in these animals were not remedied by adrenal cortical hormone therapy. On the other hand, injection of extracts of the hypophysis relieved the observed deficiencies. It is therefore concluded that hypophyseal insufficiency is induced by a chronic adrenal insufficiency and that this secondary hypophyseal dysfunction is responsible for the observed deficiencies.—A. G.

The adrenin content of the adrenals in dogs after destruction of the dorso-lumbar and sacral segments of the spinal cord (Sur la teneur en adrénaline des capsules surrénales après la destruction de la moelle dorso-lombo-sacrée chez le chien). Hermann, H., J. Dechaume and G. Morin, Compt. rend. Soc. de biol. **121:** 1000. 1936.

In 3 dogs which lived for from 1½ to 14 months after destruction of the lower cord, the adrenals did not show any marked histological change. The adrenin content was reduced.—J. C. D.

The metabolism of tissues excised from adrenalectomized rats. Himwich, H. E., J. F. Fazikas, S. B. Barker and M. H. Hurlburt, Am. J. Physiol. **110:** 348. 1934.

Numerous observations have been made with the aid of the Warburg technique of the respiratory metabolism of organs: brain, testicle, diaphragm, liver, kidney, excised from adrenalectomized rats, both in extremis and apparently in good condition as well as of control animals. The respiratory metabolism of the liver, kidney and diaphragm was unaltered by adrenalectomy. The testicle and the brain, however, exhibited a depressed oxygen consumption in the absence of glucose. The brain, moreover, suffered a decrease of respiratory quotient. In the presence of glucose, the oxygen uptake of cerebral cortex of the rats in extremis did not rise to the level of that of the normal controls with the substrate. This cannot be imputed to inability of the brain to oxidize carbohydrate. The present observations of the various excised tissues disclose no such inability. Changes of cerebral metabolism similar to those produced by adrenalectomy also occurred in rats subjected to transection of the cord and hemorrhage over a period of several hours. It is concluded that depression of metabolism is produced by lack of energy obtained from the blood constituents, glucose and O_2. This depression becomes an integral part of the syndrome of acute adrenal insufficiency. The results indicate, moreover, that the cerebral changes are, to a certain extent, irreversible and may be a cause of failure of treatment. Furthermore, the depressed metabolism is not peculiar to acute cortical adrenal insufficiency, but may occur in any conditions involving low blood pressure over a period of hours.—Authors' Summary.

Adrenal cortex extract. Kendall, E. C., J.A.M.A. **105:** 1486. 1935.

About 18 months ago the separation of a crystalline fraction was reported which possessed the essential physiologic activity in dogs and patients. This was given with a diet that contained sodium chloride in amounts slightly higher than normal. Three patients have been maintained with the crystalline material for periods of from 14 to 36 days. When the crystals were withdrawn and the administration of the same amount of salt was continued, definite symptoms of adrenal deficiency developed. These results, and similar experiments on adrenalectomized dogs with the addition of sodium chloride showed that the typical symptoms which followed the removal of the adrenals could be controlled by the crystalline fraction separated from protein, epinephrine, phospholipids and all other substances of either an acid or a basic nature. The author discusses the function of adrenal cortex extract, and its standardization, physiologic activity, the relation between adrenal cortex extract and thyroxine and the clinical results.—Abst., A.M.A. Service (Abbreviated).

Action of atropin on adrenal secretion produced by splanchnic stimulation, nicotin and quaternary ammoniums (Acción de la atropina sobre la secreción de adrenalina producida por la excitación del esplácnico, la nicotina y los amonios cuaternarios). Lewis, J. T. and F. P. Ludueña, Rev. Soc. argent. de biol. **10:** 105. 1934.

Intravenous injection of 5 mg. of atropin sulphate per kg. of weight suppresses or considerably diminishes adrenal secretion produced by splanchnic stimulation as determined by the adreno-jugular transfusion method in dogs. The effect is transitory, 15 to 20 minutes later a small secretion can be obtained that can be suppressed by further doses of atropin. Atropin does not suppress the adrenal secretion produced by nicotin and quaternary ammonium salts with nicotin action (tetramethylammonium and p-oxy-phenyl-ethyl-trimethylammonium iodides.) The increase in blood pressure produced by splanchnic stimulation is not suppressed by these doses of atropin.—J. T. L.

The occurrence and role of adrenalin in the follicle solution in the ovary (Uber das Vorkimmen und die Rolle des Adrenalins in der Follikelflussigkeit im Ovarium). Macchiarulo, O., Arch. f. Gynäk. **155:** 335. 1935. Abst., Chem. Absts. **29:** 834.

The author uses the method of Viale to obtain chemical proof of traces of adrenalin in the follicle solution. A further biological proof for the presence of adrenalin was the behavior of the isolated intestine of the guinea-pig.

Adrenal syndrome with arterial hypertension (Nebennierenrinden Syndrom mit arterieller Hypertension). Mainzer, F., Acta med. Scandinav. **87:** 50. 1935.

After a thorough review of the literature, the authors described a case of persistent hypertension (220 mm. Hg.) in a 9 year old boy which resulted in a cerebral hemorrhage and hemiplegia. The boy also exhibited an enlarged penis as well as the hair distribution and increased body size characteristic of pubertas praecox. These changes were attributable to a tumor of the left adrenal gland as evidenced by x-ray. The cause of the elevated blood pressure was postulated as being due to a substance not adrenalin present in adrenal cortex.—J. M. L.

Effects of glucose on metabolism of adrenalectomized dogs (Effects du glucose sur les échanges respiratoires des chiens privés de surrénales). Morales, A., Compt. rend. Soc. de biol. **120:** 272. 1935.

Parenteral or oral administration of glucose to adrenalectomized dogs produced a sustained augmentation of blood sugar and of the respiratory quotient.—E. Cutuly.

Effect of glucose on respiratory metabolism of adrenalectomized dogs (La acción de la glucosa sobre el intercambio respiratorio de los perros suprarrenoprivos). Morales, E. A., Rev. Soc. argent. de biol. **11:** 125. 1935.

Survival after total adrenalectomy varied from 36 to 152 hours. The blood sugar and respiratory exchanges of 5 dogs were determined before and for a period of 5 hours after administration of 2 grams glucose per kg. The same determinations were made at varying intervals after total adrenalectomy. Basal glycemia was found normal up to 8-10 hrs. before death except in 1 case, in which a low value (61 mg. %) was found. After glucose administration

hyperglycemia was prolonged when adrenal insufficiency was present. Basal metabolism decreased progressively after operation in 30-40%. The basal R.Q. also decreased from 0.60-0.72 to 0.63-0.64. The administration of glucose increases the respiratory exchanges and the R.Q. in adrenalectomized dogs and these remain above the basal level for a more prolonged period than before operation. Adrenalectomized dogs can burn sugar even in marked adrenal insufficiency.—J. T. L.

What part of the melanophore system in Fundulus is acted upon by adrenalin? Parker, G. H., J. Cell. & Comp. Physiol. **5:** 311. 1935.

The dispersing as well as the concentrating melanophore nerve fibers after having been cut in the tail of Fundulus can be shown to be fully degenerated in about 12 days. The regeneration of these fibers is complete in about 25 days. Hence shortly after 12 days the melanophores in the tail of Fundulus are fully denervated. At this time such melanophores concentrate their pigment to adrenalin. Adrenalin must therefore act directly on the melanophore.—Author's Abst.

Incretory vacuoles in the adrenalinogenous cells of the medulla of guinea pig adrenals (Sur les vacuoles incrétoires dans les cellules adrénalinogènes de la zone médullaire des glandes surrénales du cobaye). Pawlikowski, T., Compt. rend. Soc. de biol. **120:** 469. 1935.

A group of guinea pigs was bled after which physiological saline solution was passed through their circulatory systems. A second (control) group was bled without lavage. The adrenal glands were fixed in Helly-Maximow fluid, chromated, and studied cytologically. Vacuoles in the medullary cells of the glands of the control group appeared only infrequently; when present they were small. The nuclei of most of the medullary cells were heavily chromated. Numerous and large vacuoles, usually situated adjacent to walls of blood vessels, were found in the cells of glands of the first group. Generally the nuclei of the medullary cells were only lightly chromated. The author believes that these experiments indicate that hormone appears in the nucleus; passes into the cytoplasm, where it is stored; then by cellular vacuolation penetrates the blood vessels.—E. Cutuly.

ENDOCRINE GENERAL

The effect of food and of exhaustion on the pituitary, thyroid, adrenal and thymus glands of the rat. Anderson, Dorothy H., J. Physiol. **85:** 162. 1935.

The weights of the pituitary, thyroid, adrenal and thymus glands of 9 rats given an exceptionally large meal after 24 hours' fast did not differ significantly from those of 8 rats starved for 24 hours, nor from 12 controls. The effect of forced exercise was studied on 27 male rats. They were compelled to walk 11 ft. per min. for ½ hour in a revolving cage, then rested for ½ hour. This was repeated 4 times, so that the total distance walked was 2680 ft. One group of 6 animals was sacrified at the end of the exercise period, 3 rats 8 hours later, 12 after 16 hours rest, and 6 after 40 hours. The adrenal weights showed a statistically significant increase and the thymus a decrease, while the weight changes in pituitary and thyroid were insignificant. Histologically the adrenals from the exercised rats showed a lipoid distribution that differed from the controls.—R. L. Z.

Sodium chloride crystallization in pregnant women (Cristallisation du chlorure de sodium dans le sérum des femmes gravides). Bergauer, V., V. Stich, and Z. Zlabek, Compt. rend. Soc. de biol. **119:** 1131. 1935.

Sodium chloride formed different patterns when crystallized out of blood serum depending on the presence of an excess of anterior lobe hormone or of thyroid hormone, or the two together. In 25 pregnant women the pattern was that for anterior lobe secretion until a few days before delivery. At that time it varied until a few days postpartum. Then the crystallization figure was that of anterior lobe plus thyroid hormone.—J. C. D.

Metabolic stimulants and sodium dinitrophenol. Bortz, E. L., Ann. Int. Med. **8:** 599. 1934. Abst., J.A.M.A. **104:** 74.

Sodium dinitrophenol 2-4 is capable of causing a notable reduction in weight. If used without regard to a planned diet, that is, a reduction diet of from 700 to 1,000 calaries, the

loss of weight will not be striking. Used with caution, the drug may prove to be a valuable adjunct in the treatment of persons who find it difficult or impossible to lose weight by the usual established method. Dinitrophenol acts most satisfactorily in patients with the exogenous type of obesity. Those having pituitary or gonadal glandular deficiency often tolerate the drug badly or not at all. In 2 patients having mild myxedema, a heightening of thyroid activity approaching the thyrotoxic state was observed after 3 weeks of treatment. In such an event, immediate cessation of dinitrophenol therapy is imperative. The drug is not a substitute for thyroid extract.

Hormone properties of the pineal body (Über die hormonalen Eigenschaften der Zerheldrüse). Engel, P., Wien. med. Wchnschr. **84:** 1390. 1934.

According to the author, little that is definite is known concerning the physiology or function of the pineal gland. Some believe that it is an endocrine gland and others that it is a rudimentary structure and of no importance. Some believe that it stimulates, others that it hinders sexual development. Since Marburg has pointed out the association of the tumors of the pineal gland with pubertas praecox much experimental work has been done involving extirpation, implantation and the administration of various extracts. These experiments have failed to produce uniform results. The author has found an antagonistic action on the part of the pineal extract towards the growth hormone of the anterior lobe of the hypophysis and the hormones effecting luteinization and maturation of the follicles. He suggests the possibility of assaying the antigonadotropic hormone of the pineal body by its neutralizing effect against the gonadotropic hormone of the pituitary. He found the pineal hormone in small amounts in the blood. It is insoluble in alcohol, ether and acetone, is protein-free and is not heat stable.—L. G. Rowntree.

Effect of vitamins A and C on the histological structure of endocrine glands (Influence des vitamines A et C sur l'aspect histologique des glandes endocrines). Giedosz, B., Compt. rend. Soc. de biol. **120:** 557. 1936.

Subcutaneous injections of vitamins A and C in rabbits produced the following results: increase of eosinophiles and basophiles in the anterior hypophysis; stimulation of (vitamin C), depression of (vitamin A) thyroid activity; increase in number of ovarian follicles; hypertrophy of the adrenal cortex. The probable mediation of the anterior hypophysis in effecting these alterations is discussed.—E. Cutuly.

Endocrine studies in infants and children. II. Time of onset of teething, walking and talking in endocrine disorders: Its relationship to intelligence in endocrine and non-endocrine conditions. Gordon, M. B. and L. Kuskin, J. Pediat. **7:** 89. 1935.

A statistical study of the time of onset of teething, walking and talking in endocrine disturbances and of the relationship of intelligence to the developmental rate. Of 570 children studied, 369 presented evidence of endocrine disturbance and 201 manifested some degree of mental retardation without any sign of endocrine dyscrasia. About half of the endocrine group showed normal intelligence and half, retardation. The time of onset of teething, walking and talking depends on: (1) the thyroid; (2) the growth hormone of the anterior pituitary; (3) various non-endocrine factors; (4) early mentality and chronological relationship between time of inception of the influencing factor (endocrine or non-endocrine) and the normal time of teething, walking and talking. In a mentally retarded child, the time of onset of teething, walking and talking cannot be considered a diagnostic criterion of endocrine disorder. Teething is within normal age limits in mentally normal children with adiposogenital dystrophy, thyropituitary obesity and hypothyroidism. In the pituitary group there seems to be a relationship between the time of onset of teething and general growth. Dentition is delayed to the greatest extent in anterior pituitary deficiency of growth and in childhood myxedema. Premature teething is noted in 22.4% of the pituitary cases. The time of eruption in hypothyroidism depends on the relationship between the time of inception of the dyscrasia and normal teething time and also on the mentality of the infant in the first 6 months of life. Unaided walking appears in mentally normal infants at about the same average time in all endocrine conditions except hypothyroidism, in which there is a slight delay. The onset of talking is within normal limits in mentally normal children in all pituitary conditions and is slightly delayed in hypothyroidism. Infants with mental retardation teeth, walk and talk later

than mentally normal infants in the same endocrine group. In general children with non-endocrine mental retardation teeth, walk and talk later than those with endocrine disorders.—M. B. G.

Circulatory active substances resembling adrenaline in liver extracts (Beitrag zur Aufklärung der kreislaufwirksamen (adrenalinähnlichen) Stoffe in Leberextrakten). Grabe, F., O. Krayer and K. Seelkopf, Klin. Wchnschr. **13:** 1381. 1934. Abst., Chem. Absts. **29:** 1151.

Mammalian liver tissue exposed to air for 24-48 hours after death develops a substance which can be extracted with methyl alcohol and which has an adrenaline-like effect upon the heart and circulation. Tyramine has been demonstrated in such extracts, pernicious anemia and circulatory insufficiency. Their adrenaline-like action is ascribed to this substance.

Endocrine studies in mental cases. Harris, M. M., W. A. Horwitz and S. E. Katz, Psychiat. Quart. **8:** 655. 1935.

Thirty-one mental patients were investigated regarding the effect of the administration of Antuitrin-S and of Emmenin. The series included psychoses accompanied by menstrual disturbances, involutional melancholia in the male and female, several cases of young males with hypogenital development and undescended testes and women with regular menses in whom agitation or anxiety formed part of the mental picture. The purpose of the study was to determine the effect of the prolonged daily administration of the hormone and the acute effect of varying doses. In a considerable proportion of the cases improvement was seen. For details the original should be consulted.—G. L. Banay.

Variations in blood sugar in animals and the influence of adrenaline and insulin (Einige experimentelle Untersuchungen an Tieren über die Variationem des Blutzuckers und deren Beeinflussung durch Adrenalin und Insulin). Holmgren, H., Acta med. Scandinav. suppl. **59:** 104. 1934. Abst., Chem. Absts. **29:** 3014.

The normal blood-sugar curve manifests an inherent periodicity which is independent of the food intake. This rhythm is related to the variations in liver glycogen. Experiments purporting to show a direct or indirect effect on the glycemia are dubious unless the condition of the organism and of the liver at the time is taken into consideration, because the endogenous factors are of primary significance while the exogenous factors have only a transient effect. The effect of adrenalin or insulin varies according to the time of administration in relation to this intrinsic rhythm of the hepatic function.

Endocrin therapy in a case of psychic disturbance related to the menstrual cycle. Horwitz, W. A., and M. M. Harris, J. Nerv. & Ment. Dis. **82:** 551. 1935.

A 16½ year old girl had had about 30 short psychotic episodes in the past 4 years. The first attack occurred at 12½ yrs., 4 months before menses were established. At about monthly intervals thereafter she was depressed and mildly retarded, the episodes lasting about 8 days. For the next 1½ years, similar states occurred with each menstrual period. At the age of 14, instead of recovering at the end of the usual brief depressed episode, she became noisy, excitable, hyperactive and talked incessantly for an additional 8 days. Under observation the menstrual periods occurred irregularly at intervals of 2 weeks to 2 months; each period was accompanied by a psychotic episode. In the majority of these, she was disturbed, excited and overactive and in a few depressed. Antuitrin S and theelin were given at the beginning or prior to the onset of an attack or during the psychotic state and were apparently without any effect. Since her basal metabolic rate ranged from —14 to —34, 3 grains of thyroid was given daily over a period of 2 months. During this time she had a menstrual period accompanied by very mild psychotic symptoms. When the thyroid was discontinued, the basal metabolic rate, which had risen to a normal level, again dropped to —25 and with the next period a markedly disturbed mental state lasted for about a month. Ten to 15 grains of thyroid administered daily during this psychotic period produced no beneficial effect. For 6½ months an average of 8 grains of thyroid was given daily; for the last 4 months of this period the basal metabolic rate averaged +15. During this period of 6½ months, she had 5 menstrual cycles without a psychotic episode. Thyroid was then discontinued and the psychotic episodes recurred. Thyroid therapy was reinstated. The first menstrual period following the resumption was accompanied

by a mildly disturbed state lasting about 10 days. Since then, she has had 4 more menstrual periods free from either states of depression or overactivity.—Authors' Abst.

Studies on the endocrine glands of Frizzle fowl. Landauer, W., and Sophie B. D. Aberle, Am. J. Anat. **57:** 99. 1935.

It was found that in Frizzle chickens the thyroid tends to hypertrophy, eventually leading to exhaustion atrophy. The adrenal medulla is increased in mass. The testes, at the age of 8 months, show hypertrophy and exhaustion. The ovaries always are much smaller than normal. Reciprocal skin transplants show that the frizzling of the feathers is not influenced by endocrine secretions. The changes in structure of thyroid and adrenals are explained as consequences of the disturbed heat regulation in Frizzle chickens. Newly hatched Frizzle chicks tend to have slightly larger than normal thyroids with somewhat increased activity. From data of thyroid weights of newly hatched chicks from reciprocal crosses between normal and Frizzle fowls, it seems probable that the slight degree of thyroid hypertrophy in newly hatched Frizzle chicks is caused by modifying genes accumulated in the Frizzle stock, which under unfavorable environmental conditions facilitate thyroid hypertrophy.—Author's Abst., Wistar Bib. Service.

A case of pubertas praecox (macrogenitosomia praecox) in a boy associated with a tumour in the floor of the third ventricle. Le Marquand, H. S., with a pathological report by Dorothy S. Russell, Roy. Berkshire Hosp. Rep. 1934-1935.

A case of pubertas praecox in a boy is described. Abnormal somatic and sexual development began at the age of 14 months and continued until his death at the age of 4 years and 10 months. Abnormal mental development was shown by sexual advances to adult women. The cause of death was rheumatic fever, but the significant post-mortem finding as regards the pubertas praecox was a small tumour occupying the interpeduncular space attached to the right corpus mammillare and to the tuber cinereum. The tumour appeared to be a redundant portion of brain formed by developmental error and might be called a hamartoma. The abnormal development of the bones and teeth is described. The different pathological conditions which cause pubertas praecox are discussed. Reasons are advanced for endeavoring to correlate these conditions. The hypothesis is advanced that in cases in which the pineal, the adrenal, the hypothalamus, or the gonads are involved, they each and all cause pubertas praecox by an influence on the hypothalamic centres and from thence on the pituitary.—Author's Summary.

GONADS

Ovarian follicular hormone effects on the ovaries. Allen, E., and A. W. Diddle, Am. J. Obst. & Gynec. **29:** 83. 1935.

Injections of ovarian follicular hormone (amniotin) in doses ranging from 1,265 to 1,390 R.U. over periods of 28 and 39 days, were made into immature monkeys. That this hormone treatment was effective was shown by the reddening and swelling of the "sexual skin" and by increase in size of the uterus. Ovaries were studied for possible harmful effects from this treatment. The first ovary in each animal was removed immediately after a series of injections, the second ovary after a 30-day recovery period. There was a slight decrease in size of the ovaries removed immediately after the period of injections. A month later the second ovary compared favorably with its control size (some compensatory hypertrophy may have been operative during the recovery period). Decrease in size was due to a smaller number of the largest follicles. A census of normal and atretic follicles in experimental and control animals indicated a slight increase in follicular atresia. There were present in ovaries of injected animals many small cortical follicles and comparable numbers of medium-sized normal follicles in which secretion of liqour folliculi had begun. Therefore, as far as can be ascertained histologically, there seems to be little damage to the ovaries, which would be more than temporary, from injections of follicular hormone (amniotin) in the dosage and over the intervals used in these experiments. The therapeutic use of comparable doses of this hormone in children may be prescribed without fear of harmful results upon ovaries.—Authors' Summary.

Nomenclature of corpus luteum hormone. Allen, W. M., A. Butenandt, G. W. Corner and K. H. Slotta, Science **82:** 153. 1935.

During the past year the progestational hormone has been isolated from the corpus luteum in pure form and its constitution established. Heretofore two different names have been used

for this hormone in the literature (progestin, luteosterone). For the sake of international uniformity we agree to use hereafter in the scientific literature only the name progesterone for the pure hormone. As is known, the pure hormone exists in two different forms, one melting at 128° (uncorr.) and the other at 121° (uncorr.) The higher melting form (Compound B of Wintersteiner and Allen and Compound C of Slotta, Ruschig and Fels) will be known as Alpha progesterone and the lower melting form (Compound C of Wintersteiner and Allen and Compound D of Slotta, Ruschig and Fels) as Beta progesterone. We hope that these names will be generally accepted in the scientific literature.—(Reprinted).

Studies on the physiology of lactation. Inhibition of lactation by follicular hormone (Studien zur Physiologie der Milchbildung. Ueber die Laktationshemmung durch Follikelhormon). Anselmino, K. J., and F. Hoffmann, Zentralbl. f. Gynäk. **60:** 501. 1936.

The inhibition of lactation in rats by enormous doses of follicular hormone is confirmed, but this does not occur in castrated rats. Therefore the action requires the presence of the ovaries. Since the use of large doses of follicular hormone induces corpus luteum formation, it may be that the real lactation inhibiting factor is a product of the corpus luteum. Preliminary tests with a fairly pure progestational hormone preparation gave little inhibition. There may be results from some other one of the 4 fractions known to occur in the corpus luteum extracts.—E. L. S.

Effect of sex hormone on the metabolism of the sex organs (Über Stoffwechselwirkungen von Sexualhormonen auf ihr Erfolgsorgan). Aschheim, S., and H. Gesenius, Arch. f. Gynäk. **153:** 434. 1933. Abst., Chem. Absts. **29:** 2212.

The O_2 quotient of immature mouse uterus was raised by previous injection into the animal of the urine of pregnant women. This is due to its content of estrin, not to prolan. The response is not specific, being elicited by aolan, cantharidin, yohimbine and male hormones. The injection of male hormone preparations into immature male mice increased the O_2 quotient of the testes. This effect is specific. The increases in tissue respiration occur very rapidly after the injections.

Menstrual edema: Report of case controlled by emmenin but not by theelol or theelin. Atkinson, A. J., and A. C. Ivy, J.A.M.A. **106:** 515. 1936.

The authors observed a patient with pronounced menstrual edema of long standing in which certain blood chemical studies have been made and the condition prevented by the administration of emmenin (Collip) but not by theelol or theelin. The study has extended over a period of more than one year. The administration of emmenin (12 cc. daily, 60 day oral units—Collip) in the first patient resulted in a complete disappearance of the edema, including that which persisted between menstrual periods. When the emmenin was withdrawn, the edema reappeared with the next menstrual cycle. While the patient was taking emmenin there was no significant change in the basal metabolic rate (—17.5) and the menstrual headaches did not occur. A significant change in the blood lipids was not observed during the period of study. Two other patients with a similar history of premenstrual edema have been given emmenin with subsidence of the swelling.—Abst., A.M.A. Service.

Effect of natural light on testicular growth in ducks (Influence de la lumière naturelle sur la croissance testiculaire chez le canard au cours de la reprise sexuelle saisonnière). Benoit, J., Compt. rend. Soc. de biol. **120:** 131. 1935.

Fifteen male ducks approximately 7 months old, with testes partially developed, were divided into 2 groups; 1 was exposed to natural light, the other was kept in a partially darkened room. At autopsy, 1 to 3 months later, the testes (left) of the first group were well developed, while those of the second group were small.—E. Cutuly.

Stimulation by artificial light of testicular development in ducks with sectioned optic nerves (Stimulation par la lumière artificielle du développement testiculaire chez des canards aveuglés par section du nerf optique). Benoit, J., Compt. rend. Soc. de biol. **120:** 133. 1935.

Exposure to artificial light (3 weeks or more) caused in prepuberal ducks whose optic nerves had been sectioned an accentuated development of the testes.—E. Cutuly.

Stimulation by artificial light of testicular development in ducks with enucleated eyes (Stimulation par la lumière artificielle du développement testiculaire chez des canards aveuglés par énucléation des globes oculaires). Benoit, J., Compt. rend. Soc. de biol. **120:** 136. 1935.

The marked testicular development which occurs in prepuberal ducks exposed to artificial light for about 3 weeks was not prevented by excision of both eyes and closure of the eyelids. The results in this and preceding experiments are discussed.—E. Cutuly.

Induction of sexual maturity and ovulation in domestic ducks by exposure to light (Maturité sexuelle et ponte obtenues chez la cane domestique par l'éclairement artificiel). Benoit, J., Compt. rend. Soc. de biol. **120:** 905. 1935.

Exposure of immature ducks to artificial light caused precocious development of their ovaries, uteri, oviducts, and shell glands; ovulation also was induced. The effects produced by the anterior hypophyses of these animals when transplanted into immature mice indicated increased gonadokinetic activity.—E. Cutuly.

Androsterone, a crystalline male sex hormone. I. Isolation and purification from male urine (Über Androsteron, ein krystallisiertes männliches Sexualhormon; Isolierung und Reindarstellung aus Männerharn). Butenandt, A., and K. Tscherning, Ztschr. f. physiol. Chem. **229:** 167. 1934. Abst., Chem. Absts. **29:** 499.

It is still uncertain whether all the physiological effects attributed to the male sex hormone are due to one and the same substance or whether the complete development of all the sex charactristics represents the composite effect of several hormones. This study deals with an individual substance which is responsible for the development of secondary sex characteristics, notably the growth of comb and wattles in the rooster. The unit of activity is the quantity of substance required to produce in 4 days a 20% increase in the comb area of capons when injected subcutaneously on 2 successive days. The hormone was isolated from male urine. The androsterone obtained is undoubtedly an individual substance. It is hydrox ketone with activity of 1 capon unit per 150-200 gamma.

The use of corpus luteum in the treatment of dysmenorrhea. Campbell, R. E. and F. L. Hisaw, Am. J. Obst. & Gynec. **31:** 508. 1936.

Since estrogenic hormone excites contractility in the uterus, and corporin (progestin) exerts an antogonistic effect, the authors have used corporin, prepared by Hisaw, to treat a series of women with crampy dysmenorrhea, eliminating cases with demonstrable pelvic causes for pain. Doses employed were 5 to 8 rabbits units, given daily for 5 days before the expected flow, hypodermic injection being made with both alcoholic solutions diluted in saline, or oil solutions. Five cases are reported in detail from a series of 11 in which gratifying relief was obtained.—E. L. S.

Lutein hormone and cholesterolemia (Ormone luteinico e colesterolemia). Cioglia, L., and D. Tore, Boll. Soc. ital. biol. sper. **10:** 75. 1935. Abst., Chem. Absts. **29:** 3385.

Lutein hormone produced a moderate hypercholesterolemia in immature male and female rabbits. When injected with folliculin the hypocholesterolemic action of the latter was moderately inhibited. The antagonistic action of lutein hormone against that of prehypophyseal gonadotropic hormone was very marked, 1/8 U. of the former completely inhibiting the hypercholesterolemic effect of 40 U. of the latter.

Endocrinology of pregnancy in the cat (Contribution a l'endocrinologie de la grossesse chez la chatte). Courrier, R., and G. Gros, Compt. rend. Soc. de biol. **120:** 5. 1935.

The gestation period of cats is normally 64 days; ovulation occurs 26 or 27 hours following copulation; nidation of zygotes takes place 13 or 14 days after coitus. If a pregnant cat is spayed, or if its corpus luteum is excised before nidation has occurred or at any stage up to the 45th day after copulation, pregnancy is interrupted; after the 49th day pregnancy is unaffected. Depending on the stage at which pregnancy has been interrupted by spaying, the ova are resorbed in situ or the fetuses are aborted; in the latter case, the placentae frequently remain undisturbed. The assumption of the rôle of the corpus luteum by the placenta is discussed.—E. Cutuly.

Effect of folliculin on the gestating cat (Action de la folliculine chez la chatte gestante). Courrier, R., and G. Gros, Compt. rend. Soc. de biol. **120:** 8. 1935.

Folliculin in doses ranging from 500 to 1600 R.U. was administered to cats previously mated. When treatment was begun before nidation of the ovum had occurred, pregnancy was interrupted without difficulty; in later stages of pregnancy abortion could be produced only at certain stages and with large doses of hormone. Occasionally folliculin brought pregnant cats into heat without causing abortion.—E. Cutuly.

Experimental production by oestrin of pituitary tumors with hypopituitarism and of mammary cancer. Cramer, W., and E. S. Horning, Lancet **230:** 247. 1936.

Normal and castrated male and female mice were subjected to prolonged influence of oestrin administered by painting the skin twice weekly with 0.01% solution of oestrin in chloroform. Two different strain of mice were used; (1) mixed strain with low incidence of spontaneous mammary tumors, and (2) specially inbred strain with very high incidence (about 70%) of spontaneous mammary tumors. As a result of these experimental procedures 5 males of high cancer strain developed mammary cancer, 2 with tumors of the right and left axilla. The first tumor appeared after 16 weeks and the last 21 weeks after the application of the oestrin. In this strain none of the females developed a tumor in 6 months of treatment with oestrin. Of the mixed strains neither males nor females developed tumors. Oestrin painted mice also showed extensive changes in the uterus and vagina in females and atrophy of testicles in males. The process of cell division in the testis appeared to be arrested. Noticeable too, was the presence of general cachexia in mice treated over a long period of time.—L. B. S.

The morphogenic action of folliculin on the testicular ridge in chickens (Sur les effets morphogénétiques de la folliculine dans l'ébauche testiculaire du poulet). Dantchakoff, V., Compt. rend. Soc. de biol. **119:** 1117. 1935.

Folliculin in oil was introduced into the allantoic sacs of 162 embryo chicks of 4 days' incubation age. It did not influence the females but in the males produced a change in development so that the gonads became female in character; the right remained small, as in the normal female, and oviducts developed.—J. C. D.

The inability of male hormone to produce sex reversal in the female genital ridge of chickens and causes for this (Sur l'inefficacité de l'hormone male a produire une inversion sexuelle des ébauches gonadiques femelles du poulet et sur ses causes). Dantchakoff, V., Compt. rend. Soc. de biol. **119:** 1120. 1935.

Injections of male hormone into the allantoic sacs of embryo chicks 4 days of incubation age or older failed to influence the development of the gonads in the female. Folliculin reversed the sex in male chicks. These opposite results are due to the different histological arrangements of the sex cells in the gonads of the two sexes.—J. C. D.

Effect of follicular hormone on ovary and anterior hypophysis of the rat (Action de fortes doses d'hormone folliculaire sur la structure de l'ovaire et du lobe antérieur de l'hypophyse chez le rat blanc). Desclin, L., Compt. rend. Soc. de biol. **120:** 526. 1935.

In immature rats a single injection of 500 R. U. of dimenformon induced corpus luteum formation in the ovaries. The ovaries of adult rats treated with 100 R. U. of progynon B for 15 days revealed a moderate number of corpora lutea comparable in size to those of pregnancy. The anterior lobe of the pituitary of all the treated animals was characterized by diminution of eosinophiles, predominence of small basophiles, and presence of colloid in the residual cleft.—E. Cutuly.

Further studies on the developmental mechanism of the physiological condition in the genital tract (Weitere Studien über den Entsteheungsmechanismus des physiologischen Genitalmilieus). L. v. Dobszay, Zentralbl. f. Gynäk. **60:** 707. 1936.

Previous work had shown a transitory desquamation of vaginal epithelium containing glycogen, with acid secretion and the presence of the Döderlein flora in the vagina of the new born girl, under the influence of the maternal estrin. This is now extended to the production of a similar picture in nursing girls, the vaginal change being produced by injection of 100,000 to 200,000 mouse units of estrin in 4-5 days. The vaginal secretion was collected by a gauze

tampon, which was extracted with alcohol for microscopic study of cells, or with water for determinations of acids and enzymes. Results show that there is an early production of glycogen, of acids, with lower pH, and that this precedes the appearance of the Döderlein flora. They also find that other vaginal flora will simulate the Döderlein in the further fermentation of the secretion with intensification of the acidity. There is glycogenase in the secretion of the vagina without the presence of any flora.—E. L. S.

The effect of prolactin on the estrus cycle of nonparous mice. Dresel, I., Science **82:** 173. 1935.

The estrus cycle of 30 mature young female mice was studied over a period of one month. At the end of that time, 14 mice, whose cycles had been absolutely regular, were selected. These 14 mice were injected subcutaneously with one bird unit of prolactin daily, over a period of 30 days. Daily examinations showed that no changes occurred in the mammary glands as the result of the prolactin. Twenty-four hours after the prolactin injections were begun, the estrum became suspended. Daily vaginal smears from all mice showed no estrum during the following 20 to 25 days. It reappeared after this period of 20 to 25 days in spite of further continued injections of prolactin, and was markedly prolonged, continuing from 4 to 8 days. During these days the vaginal smears showed only non-nucleated epithelial cells.—Author's Abst.

On the presence of a substance antagonistic to estrogenic hormone in the urine of certain women (Sur la présence, dans les urines de certaines femmes, d'une substance antagoniste des hormones oestrales). Durupt, A., J. Lagarde and P. Bregou, Compt. rend. Soc. de biol. **120:** 852. 1935.

Urine obtained from a woman showing signs of ovarian insufficiency gave a negative Aschheim-Zondek test for estrogenic hormone. Mixtures of this urine with pregnancy urine of known estrogenic activity also gave negative tests; dilution was not responsible for this inhibiting effect, as similar mixtures of water and pregnancy urine elicited a positive response. —E. Cutuly.

Studies on the hormone of the corpus luteum (Etudes sur l'hormone du corps jaune). Fels, E., Compt. rend. Soc. de biol. **120:** 730. 1935.

Two crystalline forms of the corpus luteum hormone are recognized by the author, luteosteron C and D. Mixtures of these 2 forms injected into rabbits produced an augmented reaction, greater than their added effects when injected separately. Progesterone (a fusion of the terms progestin and luteosteron) had no effect on the testes of immature rats and was unable to prevent the appearance of histological alterations in the anterior pituitary of spayed rats.—E. Cutuly.

The question of the biological basis for the periodic fertility or sterility of women (Zur Frage der biologischen Grundlagen der periodischen Frucht- bzw. Unfruchtbarkeit der Frau). Glaser, E., and O. Haempel, Zentralbl. f. Gynäk. **60:** 702. 1936.

The method depends on stimulating the mating color in male bitterlings by injecting concentrated extracts. It is said to be more specific and dependable as an indicator of male hormone than the comb or plumage tests in use. The test also responds to corpus luteum material (referred to as the male hormone component in women). Examination of urine samples showed significant amounts present in a 13 year old girl and in women in the climacteric. Study of 24 hour samples from a single woman with 21 to 27 day cycles, followed through several cycles, showed variations from 6440 to 197 'units', with maxima during the first 2 weeks of the cycle and marked drop in the last 2 weeks. The authors suggest the drop is due to utilization of the hormone in the uterine changes. There follows much polemic with Knaus et al. about cyclic fertility and the factors influencing the uterus.—E. L. S.

Effect of male hormone on the genital organs of young male dogs (Action de l'hormone mâle sur les organes génitaux de jeunes chiens mâles). Itho, M. and K. Tsurigiro, Compt. rend. Soc. de biol. **120:** 678. 1935.

Two of 4 littermate pups 1 month old were injected intramuscularly daily for 3 and 6 weeks, respectively, with 50 mg. of male hormone obtained from human urine. With the exception of the testes which were retarded in development, all of the genital organs of the

treated pups showed a marked acceleration of growth and development as compared with those of the controls.—E. Cutuly.

Progressive modifications in the structure of the uterus and tubes of rabbits injected from birth with repeated doses of oestrone (Modifications progressives de la structure du conduit tubo-utérin, chez de lapines soumises, a partir de la naissance, a des injections répétées d'oestrone (folliculine)). Lacassagne, A., Compt. rend. Soc. de biol. **120:** 685. 1935.

Large doses of folliculin administered to female rabbits at weekly intervals from the day of their birth, for various periods of time extending over more than 2 years, produced fibromyomatous transformations of the uterus and adenomatous proliferation of the cervix. The association of the follicular hormone with pathological conditions of the uterus is discussed.—E. Cutuly.

The action of the ovarian hormones on the uterine muscle measured in vivo and in vitro. Robson, J. M., J. Physiol. **85:** 145. 1935.

To determine the significance of studies on isolated uterine muscles, simultaneous tests were made on the uterine horns of 27 rabbits. One horn was used for in vitro studies and the other in vivo. Twenty animals had been hypophysectomized to eliminate the natural oxytocic factor and 6 were bi-laterally ovariectomized. They were treated with oestrin or various combinations of oestrin plus corpus luteum to give uteri with different reactivities. The inhibitory action of luteal hormone on spontaneous contractions was only observed in vivo. The increased rhythmic activity following oestrin was generally the same in both horns, as was the reactivity to oxytocin. The exceptions were explained by occasional differences in the reaction of circular and longitudinal muscles, and differences in reactivity of different parts of the same uterus which have been shown to exist when both horns were studied by the same method. The author concludes that the results obtained with the longitudinal muscle in vitro offer, in the great majority of experiments, an accurate indication of the state of the uterus in situ.—R. L. Z.

Absorption and elimination of folliculin in the human organism. Folliculin content of food (Resorption und Ausscheidung des Follikulins im menschlichen Organismus; Follikulingehalt der Nahrung). Eng, H., Biochem. Ztschr. **271:** 370. 1934. Abst., Chem. Absts. **29:** 2217.

Although the folliculin content of foods is insignificant, the total daily consumption is relatively large. In one experiment, under the strictest conditions, the consumption of folliculin with the food is estimated as 300-400 mouse units (M.U.) per day. The amount in the urine (3-24 M.U.) and in the feces (3-57 M.U.) corresponds to that ordinarily found in normal, adult men, so that this excreted folliculin must be derived from the food.

Influence of the corpus luteum on the glycogen content of the liver (Einfluss des Corpus luteum auf den Glykogen-Gehalt der Leber). Engelhart, E. and O. Riml, Klin. Wchnschr. **13:** 101. 1934.

In female rabbits pseudo-pregnancy was induced either by a sterile male or by one intravenous injection of 10 cc. of pregnancy urine. After 10 to 12 days they were killed and the glycogen content of the liver determined immediately. Well-fed female animals served as controls. Before the glycogen determination, the control and experimental animals were starved for 24 hours. The glycogen values of the seemingly pregnant animals, which showed extremely rapid development of the corpus luteum, were 3 to 4 times as high as in the controls. In another experiment infantile guinea-pigs that received corpus luteum extract injections from 10 to 12 days showed a 30 to 60% increase of liver glycogen as compared with controls. —G. L. Banay.

Effect of progestin and estrogenic substance on human uterine contractions: Value of progestin in treatment of habitual and threatened abortion. Falls, F. H., J. E. Lackner and L. Krohn, J.A.M.A. **106:** 271. 1936.

The authors treated successfully 34 of 41 cases of threatened and habitual abortion with the corpus luteum hormone, progestin. The normal response to a distended bag varied considerably in different individuals both in frequency and in intensity of the contractions. Following

the administration of 1 cc. of solution of pituitary a marked tetany of the uterus appeared within from 3 to 5 minutes, continued for 5 to 10 minutes and was followed by regular, rhythmic, vigorous contractions which lasted for a variable length of time. During the time of the experiment the uterus usually failed to respond to a second dose of solution of pituitary. The effect of intramuscular injections of progestin in 1 Rb.U. doses on normal contractions and those contractions of the uterus produced by solution of pituitary was studied. Normal uterine contractions were completely inhibited in the great majority of cases. The effect began to show itself in from 5 to 10 minutes and lasted for the entire 2 or 3 hours during which tracings were recorded. In most instances the inhibitory effect obtained consisted of completely suspending all motility of the uterus. Solution of pituitary elicited but little or no response while the uterus was in a state of quiescence from progestin. If progestin was administered during a reaction produced by solution of pituitary, contractions disappeared in from 10 to 20 minutes. Attempts to sensitize the uterus to solution of pituitary with estrogenic substance failed during the period in which the uterus was under the influence of progestin.—Abst., A.M.A. Service.

Biology and technic of the Allen-Doisy test (Beitrag zur Biologie und Technik des Allen-Doisy-testes). Gad-Anderesen, K., and E. Jarlov, Acta med. Scandinav. **84:** 224. 1934. Abst., Chem. Absts. **29:** 2217.

Preparation of vaginal smears before castration or before administering the injection to the castrated mice is superfluous. Mice used for standardization should not be used again before the elapse of 6 weeks.

Diagnosis of pregnancy in mares (Diagnostic de la gestation chez la jument). Gallot, G., and G. Roussel, Compt. rend. Soc. de biol. **119:** 1249. 1935.

Kober described a green flourescence, seen after hydrolyzing urine from pregnant mares with sulphuric acid and then extracting with chloroform. This reaction was tested on 100 pregnant mares and 100 non-pregnant controls. Between the 4th and 10th month of gestation the results were 100% correct. The reaction depended on the folliculin content.—J. C. D.

The site of formation of the sexual hormone in the normal gravid female organism in the light of the hormone analysis of the castrate gravid state (Die Produktionsstatten der Sexualhormone im normalen gradiven weiblichen Organismus im Lichte der Hormonanalyse des ovariopriven graviden Zustandes). Guldberg, E., Acta obst. et gynec. Scandinav. **15:** 343. 1935.

Following an extensive review of the literature the findings in one case, castrated on the 113th day of pregnancy, were presented. In 6 examinations of blood and urine during the next 100 days, the amount of folliculin and gonadotropic hormone was normal for pregnancy. The gonadotropic hormone disappeared rapidly in the urine during the first few days of the puerperium but reappeared on the 28th day. During pregnancy and puerperium, the gonadotropic hormones produced a different type of reaction (a chorion type), than those excreted after the 28th day (an anterior lobe type). The anterior lobe type of reaction obtained with gonadotropic hormones excreted in the castrated state was not ascribed to the removal of the ovaries as such, but to the absence of subordinated sexual hormones such as folliculin or corpus luteum hormone or perhaps both. Demonstrable quantities of corpus luteum hormone as well as folliculin and the gonadotropic hormones were found in the placenta.—M. R. White.

Hyperplasia of endometrium: a study of endometrium after treatment. Hamblen, E. C., and W. L. Thomas, Jr., South. M. J. **29:** 269. 1936.

Endometria were obtained by curettement and punch biopsy from 27 patients varying in age from 14 to 34 years, who had been under observation for from 2 months to 2 years. The treatments for hyperplasia of the endometrium included use of anterior pituitary-like substance from pregnancy urine extracts, gonadotropic extract of anterior lobe pituitary, thyroid extract, progestin, snake venom and low-dosage radiation of the ovaries and of pituitary. There was no evidence of progestational proliferation, except in one instance following progestin therapy.—Author's Summary.

The physiology of menstruation in Macacus rhesus monkeys. Hisaw, F. L., Am. J. Obst. & Gynec. **29:** 638. 1935.

Over 40 monkeys were used in experiments dealing with the action of estrin and corporin on the endometrium after castration, and those in which endometrial modifications were in-

duced through the action of anterior pituitary preparations on the ovaries. The estrin effects are characteristic of the first half or follicular part of the cycle and corporin the last half or lutein part. It was found that postoperative menses can be inhibited by estrin following oophorectomy during the follicular half of the cycle but this effect is progressively more difficult to secure with the development of the lutein condition. When estrin is administered for an extended period following castration atrophy of the endometrium there is first marked mitotic activity which gradually subsides and may be almost absent by the end of 30 days. Such endometrium will bleed if estrin injections are discontinued or the dosage materially lowered and may even do so during treatment when injections are continued for a long time. Corporin will inhibit postcastration bleeding of either a follicular or a lutein endometrium and will also inhibit bleeding which invariably follows discontinuance of a prolonged estrin treatment. When corporin is administered for an extended period, following an estrin treatment, the endometrium develops the modifications found in the lutein half of the normal cycle, and the glands finally become exhausted. Few or no mitoses are found and this is especially true of well-developed premenstrual conditions. Corporin can also produce its characteristic effects, without previous estrin stimulation, in endometriums which have undergone castration atrophy. When corporin and estrin are injected simultaneously ½ Rb. U. of corporin is at least partially effective in the presence of 100 R.U. of estrin. Epithelial proliferations from the surface mucosa and distal portion of the glands were found in several instances following traumatization, as a result of biopsy, during the presecretory corporin phase of experimental cycles.—Author's Summary (Abbreviated).

Metabolism of women during the reproductive cycle. VII. Utilization of inorganic elements (a continuous case study of a multipara). Hummel, F. C., H. R. Sternberger, H. A. Hunscher and I. G. Macy, J. Nutrition **11:** 235. 1936.

Data are given on the progressive chemical exchange of the acid-base minerals during the final 145 days of pregnancy through the 10th to the 53rd day postpartum by a multipara when she was eating a generous and well-balanced diet. Continuous metabolic balances for calcium, magnesium, sodium, potassium, chlorine, phosphorus and sulfur showed the mean daily balances in gestation to be 0.37, 0.11, 0.56, 1.40, 0.60, 0.26 and 0.34 gm., respectively, and in lactation to be —0.48, —0.03, 0.47, 0.87, 0.39, —0.37 and —0.22 gm., respectively. The acid-base balances in pregnancy showed a mean daily retention of 347 cc., 0.1 N base which increased to 471 cc., 0.1 N base in lactation. On a maternal diet that was not only abundant in all the known nutritive essentials including minerals and vitamin D but in the proportions known to be conducive to a high maternal storage the present subject enjoyed excellent health at all times and was able to bear and rear an infant who showed no clinical or roentgenographic manifestations of rickets although it never received any direct administration of vitamin D up to the eighth month of life other than that contained in its own mother's milk.—Authors' Abst., Wistar Bib. Service.

Comparative investigation of the testes in different psychoses. Ihara, K., Juzenkai Zasshi **37:** Abst. Sec. 23. 1932. Abst. Japanese J. M. Sc. **3:** 23. 1935.

The author collected the testes in 13 cases of comparatively recent dementia praecox (16 to 28 years), the patients being free from other organic diseases. These were compared histologically with the testes of 11 patients suffering from other psychoses. In dementia praecox well marked degenerative processes in the testis were seen. The spermatoblasts showed primary atrophy without any vascular changes. The cell formation was retarded and the secretion of spermatozoa often fully obstructed. The walls of the testicular canal were thick and the lumen narrow. The lipoids in the interstitial cells were scarce or entirely lacking. Practically the same findings were made in idiocy. In other psychoses practically no changes were found.—Tr. by G. L. Banay.

The biochemistry and physiological significance of the male sex hormone. Koch, F. C., J. Urol. **35:** 382. 1936.

The history of the isolation of various forms of the male sex hormone is described together with their names and chemical formulæ. Testosterone which has been isolated from the testis in pure form and also synthesized is the most active. The author then discusses the confusing situation in regard to the control of the pituitary by the male sex hormone. Whether the variable effect on the pituitary and the accessory sex glands is due to a difference in susceptibility or whether there is a second testicular hormone is not yet clear. The daily rate of

excretion of male hormone in bird units and of oestrogenic hormone (folliculin) in rat units was followed in a group of cases. There were 6 normal men, 5 men with defective testes, 4 normal women and 4 women suffering from hypertrichosis. All showed a high degree of variability in both the absolute and relative amounts of these hormones excreted. In normal males the male hormone equalled or exceeded the oestrogenic, in males with defective testes this ratio was not changed but the absolute amounts were decreased. In normal females the output of oestrogenic hormone exceeded that of male hormone while in the hypertrichosis cases the ratio was reversed. The paper closes with the account of 2 cases, males 21 and 26 years old, where testicular deficiency was treated by injections of male sex hormone. There was some improvement but most of it was temporary.—J. C. D.

Effect of estrone on normal and castrated male rats. Korenchevsky, V., and Marjorie Dennison, Biochem. J. **28:** 1474. 1934. Abst., Chem. Absts. **29:** 3386.

Estrone has a definite effect on the gain in body weight, the weight of the sexual organs, the adrenals and the hypophysis of male rats. The presence of estrin in the male organism has therefore a definite effect on some of the organs which are also influenced by testicular hormone.

Gynecomastia, virginal hypertrophy and fibroadenomas of the breast. Lewis, D. and C. F. Geschickter, Ann. Surg. **100:** 779. 1934.

Bilateral changes were produced experimentally in the size of breasts and nipples in male monkeys (2 cebus and 5 macacus) by injections of oestrin in varying amounts from 2,000 to 5,000 R.U. The most positive results were obtained when small doses were given daily over long periods. Similar changes occurred in 1 castrated monkey. The enlargement had a tendency to regress at the end of 2 weeks. In 4 male monkeys (1 cebus and 3 macacus) in which one or both testicles were present, a condition resembling, but not identical with, gynecomastia was produced by injections of A.P.L. hormone obtained from pregnancy urine. The dose varied between 7,000 and 20,000 R.U.—M. O. L.

Experimental basis for the treatment of menopausal disturbances with diiodotyrosine (Schilddrüse und Ovarium. Experimentelle Grundlagen für die Dijodtyrosinbehandlung klimakterischer Beschwerden). Loeser, A., Klin. Wchnschr. **14:** 4. 1935.

Castration of 200 to 300 gm. guinea pigs caused parenchymal hypertrophy and hyperplasia of the thyroid within 3 weeks after operation. Twelve castrated animals receiving diiodotyrosine postoperatively for 19 days failed to show these thyroid changes. Assays on 6 normal guinea pigs showed that the thyreotropic activity of pituitaries removed from iodin-treated castrated animals was less than that found for glands taken from untreated castrated animals. Loeser concludes: that castration results in hyperfunction of the thyroid by stimulating the anterior hypophysis to increased thyreotropic activity; that diiodotyrosine prevents the thyroid hyperfunction by directly depressing the thyreotropic activity of the pituitary; that these laboratory experiments confirm Sehrt's clinical observations on the value of diiodotyrosine in menopausal emotional disturbances.—H. B. F.

Effect of sex hormone on blood coagulation and its variations in hormonal hermaphrodism and experimental sex changes (Die Beeinflussung der Koagulationszeit des Blutes durch das Sexualhormon und ihre Variationen bei hormonalem Hermaphroditismus und experimentellem Geschlechtswechsel). Lundberg, E., Acta med. Scandinav. Suppl. **59:** 432. 1934. Abst., Chem. Absts. **29:** 3014.

Definite variations in the blood coagulation are observed in animals according to their sex hormone condition. It is suggested that in male homosexuals it is best to administer preparations affecting the female and not the male sex organs, that is, folliculin, in large doses and over a long period of time. In homosexual females the situation is more difficult.

Observations on ovulation in the rabbit. Markee, J. E., and J. S. Hinsey, Anat. Rec. **64:** 309. 1936.

The phenomenon of ovulation has been followed in the living animal and histological observations have been made on 50 pairs of serially-sectioned rabbit ovaries in which ovulation had occurred. Attention was focused upon changes in the vascular bed about the follicle and the histology of the rupture point. Vascular changes and other events in ovulation showed

no observable variations in ovaries in which the connections with the central nervous system were completely severed or in ones which had been hypophysectomized 2 to 3 hours before the injection of pregnancy urine. Graphic reconstructions of 90 rupture points in 24 ovaries showed them to be oval-shaped with a mean area of 0.062 sq.mm. No evidences of serrations indicating slit-like tearings of the ovarian wall were present. The observations indicate that a portion of the ovarian wall is actually removed at the time rupture starts. Three-dimensional reconstructions of the theca, granulosa and contents of the antrum are described for mature follicles before and after rupture. One of these from an ovary that had ruptured in the fixative shows the thread-like projection of follicular liquor with germinal epithelial cells at its tip and the ovum near the tip. Another from a ruptured follicle contains an ovum which had failed to leave the antrum. This observation is significant in an explanation of primary ovarian pregnancy.—Authors' Abst., Wistar Bib. Service.

HYPOPHYSIS

Effect of extracts of anterior pituitary and of prolan on lactation. Asimoff, G., V. Ptchelina and N. Parijsskaya, *In* The Problems of Zootechnical and Experimental Endocrinology (Transactions of the Endocrinological Laboratory of the All-Union Institut of Animal Breeding) **1:** 85. 1934.

Prolan is able to induce (in certain doses) proliferation of mammary glands as well as secretion of colostrum in rabbits and dogs. The authors had no success in inducing milk-secretion in these animals by means of prolan-treatment. Extracts of the anterior lobe of the pituitary gland induced lactation in normal (non-castrated), as well as in castrated dogs. It was impossible to successively induce lactation by means of pituitary more than twice in either rabbits or dogs, (normal or castrated). It was noted that a periodical spontaneous lactation, occurring in experimental rabbits, might obscure the results of the experiments. Injection of alkaline extracts of the anterior lobe of the pituitary gland was quite harmless for the rabbits, as well as for the offspring.—Authors' Summary.

Effect of pregnyl on pregnant rats (Die Wirkung von Pregnyl bei Schwangeren Ratten). Bergmann, F., Acta Brevia Neerland. Physiol., Pharmacol., Microbiol. **4:** 20. 1934. Abst., Chem. Absts. **29:** 3383.

Observations were recorded on 20 pregnant rats treated with the luteinizing hormone of the hypophysis. The ovaries during heat contained large follicles (which persist till the end of pregnancy), blood-filled and ripe follicles and fresh corpora lutea. Normal ovaries of equal term showed part new and part old corpora lutea. In some cases of early treatment abortion took place, though in many cases birth came at term. The festuses of pregnyl-treated rats weighed as much as those of normal mothers. The sensitiveness of the uterus to pituitrin in pregnyl-treated rats was the same as that of normal rats both in vivo and in vitro.

Glucose consumption by hypophysectomized pancreatectomized dogs (El consumo de la glucosa por los perros privados de la hipófisis y del páncreas). Biasotti, A., Rev. Soc. argent. de biol. **10:** 82. 1934.

The respiratory quotient increases after ingestion of glucose in hypophysectomized pancreatectomized dogs and does not increase in pancreatectomized dogs with an intact pituitary. Therefore glucose can be burned in the absence of insulin if the pituitary is also absent. Observation of glycosuria following glucose administration also shows that part of the sugar given is retained. The blood sugar curve following glucose injection in pancreatectomized hypophysectomized dogs shows a smaller initial increase and a faster decrease than is seen in pancreatectomized dogs; the fasting blood sugar value is also lower.—J. T. L.

Glucose tolerance of hypophysectomized dogs (Tolerancia de los perros hipofisoprivos a la glucosa). Biasotti, A., Rev. Soc. argent. de biol. **10:** 124. 1934.

Hypophysectomized dogs, 17 to 280 days after operation, received 1 gram glucose per kg. intravenously, after 18 to 24 hours' fast. The blood sugar (Hagedorn-Jensen) curve was followed for 6 hours. The initial rise (15 mg. % after injection) was slightly greater on average in the 14 hypophysectomized animals than in 10 controls, the return to the initial level was also retarded. The blood curve following intravenous glucose injection in 5 dogs with lesions of the tuber cinereum was not significantly different from the normal one. Ingestion of glucose (2 mg. per kg.) was followed by a blood sugar curve slightly lower and more

prolonged in 6 hypophysectomized animals than in 5 normal animals; it would seem that absorption by the gut was retarded.—J. T. L.

Transplantation of the hypophysis cerebri to the anterior chamber of the eye in albino rats. Buxton, C. L., Anat. Rec. **64:** 277. 1936.

Forty-two rats received hypophyseal transplants into the anterior chamber of the eye. The majority of these were autotransplants obtained by simultaneous hypophysectomy. A lesser number consisted of homotransplants into recipients both with and without concurrent or previous hypophysectomy. Of the 42 animals so prepared, 7, or 16%, showed viable transplants at the end of 3 to 6 weeks when the rats were killed. These successful transplants consisted of recognizable anterior lobe tissue, but no growth of the neurohypophysis was observed. Ample vascularization of the transplants had occurred. Evidence of takes of the transplants was based solely upon histological examination of serial sections of the eye. The number of successful takes was too small to make any statement regarding the relative viability of homo- or autotransplants.—Author's Abst., Wistar Bib. Service.

Prehypophysial gonadotropic hormone and cholesterolemia (Ormoni gonadotropic preipofisarii e colesterolemia). Cioglia, L., and D. Tore, Boll. soc. ital. biol. sper. **9:** 1249. 1934. Abst., Chem. Absts. **29:** 2215.

In normal male, female and ovariectomized rabbits the intensity of hypercholesterolemia, within certain limits, paralleled the amount of hormone administered.

Structure of the hypophysis of the guinea pig post partum (La structure de la glande pitutaire du cobaye dans le post-partum). Collin, R., and P. Florentin, Compt. rend. Soc. de biol. **120:** 143. 1935.

During gestation the anterior hypophysis of the guinea pig is markedly eosinophilic; a few days before parturition the acidophilic cells are replaced by cords and islets composed chiefly of basophilic cells. That part of the anterior lobe adjacent to the intermediate portion contains groups of large chromophobic cells and occasional basophiles. Capillaries surrounding these groups are impregnated with colloid. Within 24 hours after parturition the chromophobic masses extend throughout the anterior lobe. The intensity of coloration of the basophiles bordering capillaries seems to vary inversely with the amount of colloid present in the vessels, so that there is a gradual change from basophile to chromophobe. These observations are discussed with reference to the unitarian idea of origin of cell types in the anterior hypophysis and to the release of the lactogenic principle.—E. Cutuly.

Presence of the oxytocic, pressor and anti-diuretic factors of the posterior lobe in the cerebrospinal fluid (Nachweis der oxytocischen, blutdrucksteigernden und diureschemmenden Komponenten des Hypophysenhinterlappensekretes im Liquor cerebrospinalis). Deleonardi, S., Arch. f. exper. Path. u. Pharmakol. **180:** 135. 1936.

Experiments for demonstrating the presence of the posterior lobe factors in the cerebrospinal fluid of the dog, rabbit and man were made. The site of withdrawal of the fluid appears significant for detection of the presence of these substances. Fluid withdrawn from the cervical level in the dog and rabbit, and from the 3rd ventricle in man contained the oxytocic factor. The tests were made on strips of guinea-pig uterus. The blood pressure and diuretic factors were present in the same specimens. Lumbar punctures in man revealed only a trace of the oxytocic factor in the fluid, and no pressor or diuretic substance. The oxytocic factor of the fluid corresponded approximately to 0.4 gamma per cc. compared to fresh posterior lobe substance. Studies on the oxytocic, antidiuretic and pressor content of the posterior lobe substance were made for comparison with the results obtained with the cerebrospinal fluid. Some variations from the results reported by Burns were found in the diuresis experiments on rats. It was thought that a difference in the experimental conditions might account for these findings.—R. Cleveland.

The effect of hypophyseal hormone content on tumor growth (Die Wirkung der Hypophyseninkretion auf Geschwülste). Druckrey, H., Arch. f. exper. Path. u. Pharmakol. **180:** 367. 1936.

The experiments on the effect of the gonadotropic content of the hypophysis on the growth of the implanted Jensen Sarcoma, or Flexner-Jobling Sarcoma were made on rats. Tumors were

implanted by subcutaneous injection of 4 cc. of tumor pulp in 4 parts Ringers' solution. Normal, 'freshly castrated,' and castrates of 3, 4, 6 and 8 months' duration were used. Careful preliminary experimental work on the gonadotropic content of castrate hypophyses showed the content increased up to 7 months and then returned to normal levels. The mortality following tumor implantation into 55 freshly castrated rats were 100% within 28-35 days as contrasted with the 40 days in normal animals. In 79 castrates of 3, 6, and 8 months' duration, the mortality was 48%. A group of 'old' castrates treated with progynon and receiving tumor implants showed 69% mortality, and 23 senile rats showed an 82% mortality. Diffuse necrosis was found in the tumors of the fresh castrates. The lowest mortality rate corresponded to the highest gonadotrope content of the hypophysis indicating an inhibitory effect of the gonadotrope factor on tumor growth. The respiratory metabolic rate of the tumor tissue as determined by the Warburg method was in direct proportion to tumor growth. In normal animals tumor implantation brought about a suspension of the estral cycle, and the ovaries showed no developing follicles, corpora lutea only for a short time, and finally only atretic follicles were present. In the normal male rat implanted with the tumor the testis atrophied within a few weeks, spermatogenesis was interrupted and a sloughing of the epithelium occurred. The weights of the hypophyses and pineals of the experimental animals were recorded. The author concludes that tumor growth is influenced by the endocrines, while the tumor itself may influence the hormone content of the gland.—R. Cleveland.

Relation of hypothalamico-hypophyseal system to diabetes insipidus. Fisher, C., W. R. Ingram and S. W. Ranson, Arch. Neurol. & Psychiat. **34:** 124. 1935.

With the aid of the Horsley-Clarke stereotaxic instrument, lesions were placed in various parts of the hypothalamus in 40 adult cats. In 10 instances prolonged and permanent polyuria developed, while in 2 cats transient polyuria was observed. The transient polyuria differed from the permanent polyuria in the following respects: It develops much more rapidly and reaches a peak much sooner than does the permanent polyuria; the polyuria is primary to the polydipsia during the permanent phase, while the intake of fluid usually exceeds the output of urine on the first day of the transient phase; during the transient phase the output of urine and the intake of fluid may reach proportions never observed in the permanent phase. The polyuria and polydipsia in the 10 diabetic cats lasted from 2 to 9 months and appeared to be permanent. Repeated small doses of pitressin injected subcutaneously caused a reduction in the urine output and the fluid intake to normal levels. Permanent polyuria was found to occur only in the cases in which there was bilateral injury to the supra-optico-hypophyseal system. Such injury resulted in marked atrophy of the supra-optic nuclei and marked atrophy of the posterior lobe. Unilateral damage to this system did not cause polyuria, nor did lesions in any of the other parts of the hypothalamus. The evidence from this investigation supports the theory that diabetes insipidus is a hormonal disturbance, caused by a deficiency in the secretion of an antidiuretic principle by the posterior or the intermediate lobe of the pituitary gland. The view is set forth that the supra-optico-hypophyseal system sends secretory impulses to these divisions of the hypophysis and that damage to this system at 1 of 3 points, the nucleus, the fiber tract and the pars intermedia and pars neuralis, results in diabetes insipidus. —Authors' Summary (Abbreviated).

Differentiation of gonadotropic effects of anterior pituitary extracts. Freud, J., and E. Dingemanse, Acta Brevia Neerland. Physiol., Pharmacol., Microbiol. **4:** 54. 1934. Abst., Chem. Absts. **29:** 3384.

The sex-stimulating properties of extracts of the anterior pituitary were tested for their effect upon the weights of various organs in the rat. Preputial glands are stimulated to growth by adequate doses of all but one of the preparations. Crude alkaline extracts, neutralized NaOH extracts of acetone powder, NH_3 extracts of acetone powder and pyridine extracts of acetone powder have sex-stimulating effects. Seminal vesicles are practically unaffected by all dosages applied.

Effect of alkaline anterior pituitary extract on minerals of plasma (Acción del extracto alcalino de lóbulo anterior de hipófisis sobre las substancias minerales del plasma). Gerschman, Rebeca, and A. D. Marenzi, Rev. Soc. argent de biol. **11:** 485. 1935.

Hypophysectomy produced a significant decrease in plasma K, which did not occur after pancreatectomy or thyroidectomy but was observed in hypophysopancreatectomized dogs. Mg did not vary significantly. Ca, Mg, Na, Cl and CO_2 diminished after pancreatectomy; in

hypophyso-pancreatectomized dogs Ca, Cl and Na diminished, Mg and CO_2 did not. Diabetogenic anterior pituitary extract given in large doses produced an increase in the alkaline reserve, glycemia, Cl, Mg and inorganic P and a decrease in Cl and Na; kidney and muscle extracts did not produce these effects. Thyroidectomy did not alter the action of the extract. The increase in blood Ca produced by the extract occurred in hypophysectomised dogs but not in thyro-parathyroidectomized ones. It is deduced that the extract stimulates the parathyroid.—J. T. L.

Elimination of thyrotropic substance in urine of menopausal women (Die Ausscheidung thyreotrop wirksamer Substanz im Harn klimakterischer Frauen). Grumbrecht, P., Zentralbl. f. Gynäk. **59:** 1331. 1935. Abst., J.A.M.A. **105:** 325. (Abbreviated).

An extract prepared from the urine of climacteric women was injected into infantile rabbits; these promptly developed accelerated heart action and other symptoms. Some of the animals died; the others were killed on the third day. Histological examination of the thyroids revealed changes indicating increased functional activity. The urine of the women who were in the early stages of menopause produced less severe changes. The urine eliminated during the menstrual period likewise seemed to contain thyrotropic substance, while the urine of sexually mature women outside the menstrual period produced on changes. The author concludes that the menopausal disturbances are the result of over-functioning of the anterior lobe of the hypophysis, which is induced by the abolishment of the gonadal function. The treatment should attempt to reduce the thyrotropic action by the administration of iodine or of estrogenic substance.

Studies on gonadotropic hormones from the hypophysis and chorionic tissue with special reference to their differences. Hamburger, C., Acta path. et microbiol. Scandinav., suppl. **17:** 1. 1933. Abst., Chem. Absts. **28:** 5870.

An increased excretion of a follicle-stimulating anterior hypophyseal hormone, similar to that excreted by castrated women, was found in the urine in 75% of the cases studied, that is, in 14 male castrates (from 3 weeks to 13 years after castration) and in 15 women past menopause (59-85 years old). The urine of men or boys who were normal or diagnosed as sexually impotent or infantile did not show any increase in this hormone.

A case of Cushing's syndroma [basophile adenoma of the hypophysis] (Prípad Cushingova syndromu [basofilni adenom hypofysy]). Hora, F., Bratisl. lekár. listy, **15:** 77. 1935.

Detailed study of a typical case of Cushing's syndrome in a woman of 38 years with a basophile adenoma of the pituitary gland is reported. Sudden impairment in the third month of the disease explained by dissection as an isolated hemorrhage into the adenoma was mainly interesting. Clinical symptoms were compared with the results of pathological examination. Special attention was devoted to the disturbance of carbohydrate metabolism. Exact analysis of the case does not testify the hyperplasy of basophile cells in a Cushing's syndrome as being a primary one.

Production of lactation in dogs and bitches by anterior pituitary extract (Provocación de la secreción lactea en perros por medio del extracto ánterohipofisario). Houssay, B. A., Rev. Soc. argent. de biol. **11:** 240. 1935.

Alkaline anterior pituitary extract (Evans and Simpson's) given in repeated doses intraperitoneally produced secretion of milk in non-pregnant, non-lactating bitches. At first the mammary gland is enlarged, a serous secretion appears and finally milk is secreted. This can be obtained in normal bitches and after castration, thyroidectomy, section of both splanchnic nerves and extirpation of the abdominal sympathetic chain, hypophysectomy and castration, hypophysectomy and thyroidectomy. It cannot be obtained in young bitches before puberty. The results are more marked the larger the mammary gland is at beginning of treatment. The activity of the extract is destroyed by boiling for 15 min. but not by heating to 70° C for 15 min. The extract does not produce lactation in male dogs, but this can be produced if they are given previously a prolonged and intense treatment with oestrin sufficient to produce hypertrophy of the mammary gland. This last was seen in 1 normal, 1 castrate and 1 hypophysectomised castrate.—J. T. L.

Effect of hypophysectomy on pregnancy and lactation in the dog (Action de l'hypophysectomie sur la grossesse et la sécrétion lactée, chez la chienne). Houssay, B. A., Compt. rend. Soc. de biol. **120:** 496. 1935.

Hypophysectomy of 14 dogs in late pregnancy caused abortion or resorption of the fetuses. The dogs did not lactate after the operation, but milk secretion was induced in 3 of them by the injection of anterior pituitary extract. In 5 dogs suckling pups lactation ceased 3 to 5 days subsequent to hypophysectomy; milk secretion was induced by anterior hypophyseal extract in 2 or 3 dogs. Injury of the tuber cinereum and extirpation of the posterior lobe of dogs had no effect on pregnancy or lactation.—E. Cutuly.

Lactation induced by anterior hypophyseal extract in male dogs (Sécrétion lactée provoquée par l'extrait anté-hypophysaire chez le chien). Houssay, B. A., Compt. rend. Soc. de biol. **120:** 502. 1935.

Anterior pituitary extract was effective in inducing lactation in female dogs with well developed mammary glands and in male dogs whose mammary glands had been stimulated previously by injections of folliculin. Under similar conditions milk secretion was induced even in the absence of ovaries, testes, thyroid, and of lumbar sympathetic chains.—E. Cutuly.

Functional modifications of the hypophysis in toads following infundibulo-tubular lesions (Modificações fonctionelles de l'hypophyse après les lésions infundibulo-tubériennes chez le crapaud). Houssay, B. A., A. Biasotti, and R. Sammartino, Compt. rend. Soc. de biol. **120:** 725. 1935.

The diabetogenic action of the principal lobe of the pituitary was prevented in pancreatectomized toads whose tuber had been injured. Transplantation of the glands into hypophysectomized-pancreatectomized toads, with tuber intact, showed them to possess a diabetogenic power. The cause of the inhibition in animals with tuberal lesions was attributed to circulatory disturbances.—E. Cutuly.

Hypophysis and compensatory hypertrophy of testis in toads (Hypophyse et hypertrophie compensatrice du testicule chez le crapaud). Houssay, B. A., and J. M. Lascano Gonzalez, Compt. rend. Soc. de biol. **120:** 362. 1935.

Sub-total castration of toads with intact hypophyses was followed by compensatory hypertrophy of the testicular fragment. If the animals were hypophysectomized, the fragment became atrophic. Hypertrophy of the testicular remnant was produced in hypophysectomized animals by anterior pituitary implants.—E. Cutuly.

Diabetogenic action of anterior hypophysis independent of the suprarenals (Action diabétogène anté-hypophysaire independante des surrénales). Houssay, B. A., and L. F. Leloir, Compt. rend. Soc. de biol. **120:** 670. 1935.

Hyperglycemia was produced by injections of alkaline extracts of bovine anterior pituitary: in toads deprived of hypophysis, pancreas and adrenals; in 2 dogs from which the entire right suprarenal and the medulla of the left gland had been extirpated; in 2 dogs following sectioning of the splanchnic nerves and ablation of the lumbar sympathetic chains; in 3 bilaterally adrenalectomized dogs.—E. Cutuly.

Anterior pituitary diabetogenic activity independent of the adrenals (Acción diabetógena ántero-hipofisaria independiente de las suprarenales). Houssay, B. A., and L. F. Leloir, Rev. Soc. argent. de biol. **11:** 464. 1935.

Intraperitoneal injection of alkaline extract of the anterior pituitary produced hyperglycemia (diabetogenic effect) in dogs after extirpation of the adrenal medulla or denervation of the adrenals. In another series the right adrenal was removed and extract given until the diabetogenic effect was evident; the left adrenal was then removed, in half the animals the extract was continued and the blood sugar remained high; in the others the pituitary treatment was suspended and the blood sugar fell. In hypophyso-pancreatectomized toads the adrenals were cauterized, a glandular lobe of the pituitary was implanted or its suspension injected subcutaneously; 20 hours later the blood sugar was determined, and a significant increase was observed which was slightly less than that seen in controls with intact adrenals. The diabetogenic effect of the anterior pituitary was not due to adrenal activity.—J. T. L.

A case of Simmond's disease due to a primary tumor of Rathke's Pouch, observed over a period of twelve years. Howard, C. P., and L. F. Rhea, Internat. Clin. **1:** 1. 1936.

This is an excellent, though brief, review of the etiology of Simmond's disease with a detailed report of an interesting case. The patient was a female of 23, whose case terminated fatally. Postmortem examination revealed a group of pathological changes resulting from a Rathke Pouch tumor which, by accident of position, had pressed upon the pituitary gland, thus interfering with its physiological functions.—I. B.

A comparative study of the Kelly test and the Friedman modification of the Zondek-Aschheim Test for pregnancy. Hulpieu, H. R., J. H. Weatherby and C. G. Culbertson, J. Lab. & Clin. Med. **20:** 63. 1934.

The Kelly test for pregnancy was found to compare favorably in accuracy with the Friedman modification of the Zondek-Aschheim test in a series of 59 cases, the results having been checked against the clinical findings in each case. It appears probable that certain pathologic conditions affecting the ovaries directly may give a false positive reaction in the Kelly test, whereas the Friedman test will be negative under the same conditions. Since the injections of urine in the Kelly test are made intraperitonally, contaminated specimens are more apt to kill the experimental animals than is usually true when the injections are made intravenously, as in the Friedman test. The Kelly test was found to offer no important advantage over the Friedman test except in the lower cost and the greater ease of handling the smaller animals.—Authors' Summary.

Cushing's disease (de Giebke van Cushing). de Jongh, C. L., Nederl. tijdschr. v. geneesk. **79:** 1805. 1935.

A case report is given of a woman suffering from amenorrhea, hypertension, diabetes, advancing weakness and osteoporosis. There was no polycythemia. There was considerable gain in weight and abnormal hair growth of masculine distribution. After death none of the endocrine glands showed any anatomical lesion. Serial sections of the pituitary body were made, but no basophilic tumor was found. It is concluded that pituitary basophilism need not necessarily be the result of a basophilic tumor of the pituitary body.—L. M.

Action of gonadotropic extract of urine pregnyl on tar carcinoma. Julius, H. W., Acta Brevia Neerland. Physiol., Pharmacol., Microbiol. **4:** 74. 1934. Abst., Chem. Absts. **29:** 3392.

Mice painted with an active carcinogenic coal tar were given daily subcutaneous injections of 100 units of pregnyl for a period of 18 weeks. Since no prophylactic effect was obtained, gonadotropic extract from urine apparently had no preventive power on the development of tar carcinoma.

Mode of action of mammalian anterior hypophyseal extracts on secondary sexual characteristics of male Discoglossus (Mode d'action des extraits hypophysaires antérieurs de mammifères sur les caractères sexuels secondaires du Discoglosse mâle). Kehl, R., Compt. rend. Soc. de biol. **120:** 10. 1935.

The thumb callosities of male Discoglossus in captivity underwent a relatively slow involution of variable degree. Castration caused a rapid and complete regression of the pads. Administration of anterior pituitary extract to intact captive toads caused the pads to attain the size and appearance of those of sexually active free animals; similar treatment had no effect on toads castrated 3 months previously. The testis was, therefore, the essential intermediary through which the anterior pituitary influenced secondary sexual characters.—E. Cutuly.

Ulceration of the digestive tract following hypophysectomy. Keller, A. D., and M. C. D'Amour, Arch. Path. **21:** 185. 1936.

The temporal approach was utilized in a series of 23 dogs for the removal of the hypophysis. Of these 6 died within 48 hours after operation, 15 exhibited hypoglycemic crisis, and 2 dogs resisted this crisis. Eight dogs recovered after the administration of dextrose, but finally died. In 3 dogs of this series total pancreatectomy was performed prior to hypophysectomy. Characteristic hemorrhagic states as well as crater formation were encountered with low

irregular incidence after attempted total hypophysectomy in the dog. Typical twin duodenal ulcers were observed in 4 of the 23 dogs at death on the 4th, 9th, 15th, and 16th days after operation. In this series of 23 dogs, hemorrhage of the G. I. tract was encountered in 5 instances at death which occurred 24 hours after operation. Hemorrhagic changes were noted by bloody mucoid feces and by gross and histologic changes. In 13 dogs in which abdominal vagotomy was performed prior to hypophysectomy, no crater formation was encountered. In 13 dogs in which the abdominal portion of the chains were removed before hypophysectomy no hemorrhagic states were encountered. Multiple gastric craters, occurring predominantly in the pylorus were encountered in 5 dogs, with definite duodenal involvement in 5 instances.—L. B. S.

Pituitary hyperfunction in tuberculosis (Hypophysaire reactie bj tuverculose). Koster, L., and P. A. Heeres, Nederl. tijdschr. v. geneesk. **79:** 2336. 1935.

A syndrome occurring in tuberculosis infections is described. It is believed to be a reactive hyperfunction of the hypophysis resulting from the infection. Report is made of 6 such cases. The appearance of these patients showed some similarity to pituitary basophilism described by Cushing. The patients became obese. There was polycythemia (about 6,000,000 per cu. mm.), hypertension, diabetes, and amenorrhea. There was no hirsutism and no osteoporosis. The blood cholesterol was elevated. In 4 cases there was slight hypercalcaemia (12.16 mg. per 100 cc.). The picture had much in common with that of hyperfunction of the hypophysis and the adrenal cortex.—L. M.

The effect of luteinizing substance on the function of the lipoid containing cells of the ovary (Über die Wirkung luteinisierender Substanz auf die Funktion der lipoidhaltigen Zellen des Ovariums). Kuschinsky, G. and Tang-sü, Arch. f. exper. Path. u. Pharmakol. **179:** 717. 1935.

The effect of the luteinizing substance of pregnancy urine prolan on the ovaries of 26 very young rats was studied. The luteinizing factor (25 R. U.) was injected subcutaneously each day for 8-10 days into immature rats 7-8 days old and continued until the time of sacrifice on the 17-20th day of life. The ovarian weights were not essentially increased in the experimental animals. Histologically the ovaries showed extensive thecal luteinization. The interstitial tissue contained lipoid cells very similar in appearance to that of the thecal cells. No follicle maturation occurred. Actually the number and size of the follicles were less in the treated than in the control animals, and in 2 instances the weight was less. The weight of the uterus was greater in the experimental animals, and the vagina was opened, indicating that some ovarian secretion had been produced. A histological examination of the uterus showed a thickening of the uterine musculature, while the mucosa was not especially affected. As a means of testing for a possible corpus luteum hormone reaction in the uterus the foreign body stimulation of the uterine mucosa was used. There was only a slight local reaction to this stimulation, and no decidual formation. The authors concluded that the luteinizing factor of pregnancy urine depresses follicle maturation in infantile ovaries since the differences between the ovaries of the experimental and control animals were merely quantitative. The authors believe the lutein cells apparently produced hormone somewhat similar to the follicle hormone in its action on the uterine musculature and the vagina.—R. Cleveland.

The effect of luteinizing substance on the testis (Über die Wirkung luteinisierender Substanz auf den Hoden). Kuschinsky, G. and Tang-sü, Arch. f. exper. Path. u. Pharmakol. **179:** 722. 1935.

The authors investigated the effect of the luteinizing factor of pregnancy urine prolan on the testis of 6-8 day old rats treated daily with 25-50 R. U. of the substance over a period of 7-10 days, the animals being sacrificed on the 18-20th day of life. The testis and seminal vesicles were weighed. The testis of the experimental animal was only slightly or not at all enlarged. There was an increase of the testicular interstitial tissue which did not affect the seminiferous tubules. The great enlargement of the seminal vesicles in the experimental animals was taken as an indication of the increase of the endocrine function of the testis. The hormone produced by the increased interstitial tissue acted upon the seminal vesicles in a manner similar to that of the male sex hormone. Thus in both male and female infantile rats it would appear that the luteinizing substance of pregnancy urine did not act upon the germinal epithelium but only upon the lipoid containing cells, the interstitial tissue.—R. Cleveland.

Effect of temperature variations on secretion of thyreotrope hormone (Über den Einfluss verschiedener Temperaturen auf die Sekretion des thyreotropen Hormone). Kuschinsky, G. and Tang-sü, Arch. f. exper. Path. u. Pharmakol. **179:** 726. 1935.

The effect of 4° C and 38-40° C temperatures on the production of the thyreotrope factor of the anterior lobe was studied in white male rats under well-controlled conditions. A third group of animals kept at room temperature served as controls. The studies extended over a period of months; 112 animals were in the control group, 67 in the increased temperature group, and 67 in the group kept in the lower temperature. In the latter group the thyroid presented a picture of a resting gland after a period of 10 days, while after 24-30 days the histological picture was one characteristic of increased thyroid activity. Periods of 12, 24 and 48 hours in the cages kept at higher temperatures produced no noticeable effect upon the thyroid; changing the animals from warm to cold cages and back to warm ones produced no effect other than a tendency to the appearance of an inactive or resting gland. The actual thyreotrope content of the hypophyses of these animals was tested by implanting the hypophyses into young guinea-pigs. After 2 days in the cold cages there was no variation from the normal; 10 days in the cold produced a marked increase in the thyreotrope factor; 25-30 days in the cold produced no changes from the content of the normal hypophysis. In the warm cages (4-6 days) the thyreotrope content of the hypophysis was decreased below that of the normal; after 14-23 days there was no demonstrable amount of the thyreotrope factor present. After 26 days at the increased temperature a slight increase of the thyreotrope factor was apparent.—R. Cleveland.

Effect of removal of the hypophysis on the development of tumors in the rabbit (Influence de la privation d'hypophyse sur le développement des tumeurs chez le lapin). Lacassagne, A., and W. Nyka, Compt. rend. Soc. de biol. **121:** 822. 1936.

Three types of tumors, those from coal tar, benzopyrene, and direct inoculation of papilloma, were tested on 18 rabbits whose hypophyses had been destroyed by radium. Eighteen controls received the same treatment. The mortality rate in the hypophysectomized animals was high, but in those surviving, the tumors failed to develop, although the controls showed a high morbidity. One animals developed an oesteosarcoma of the sella turcica 15 months after radium treatment.—J. C. D.

The follicular apparatus of the ovary of the hypophysectomized immature rat and the effects of hypophyseal gonadotropic hormones on it. Lane, C. E., and R. O. Greep, Anat. Rec. **63:** 139. 1935.

Thirty-three 28-day-old female rats were hypophysectomized and killed in groups of 3 at periods ranging from 1 to 38 days after operation. Their ovaries were weighed, the follicles counted and measured. It was found that all the curves show a rise at 4 days which is followed by a uniform decrease, except for the follicular diameter curve, which never descends below 250 μ. The fourth day peak is ascribed to pituitary materials forced into the circulation of the rat during the traumatization of the gland which necessarily precedes its removal. It was found that F.S.H. always produced (1) an increase in the total follicle count—this being largely due to the formation of numerous small primary follicles; (2) a decrease of the vesicular percentage. Luteinizing hormone administration, on the other hand, was without effect on the total follicle count, but increased the vesicular percentage from 35 to 112% over the control in the immediate and delayed treatments, respectively.—Authors' Abst., Wistar Bib. Service.

Pituitary infarcts after producing tuberal lesions in the toad (Infartos hipofisarios por lesiones tuberianas en el sapo). Lascano Gonzalez, J. M. Rev. Soc. argent. de biol. **11:** 309. 1935.

The tuberal region was cauterized between the optic chiasma and the pituitary, so that most of the lobus infundibularis was destroyed. The pituitary appeared intact on macroscopical examination, but microscopically in every case destructive lesions were found in the central part of the glandular lobe, which are due to alterations in the circulation; they constitute an ischem infarct. These increase in intensity and extension for a week after the operation; at about the 11th day they cease to grow and afterwards healing commences with neoformation of glandular tissue, the connective tissue scar being of little importance. The cells of regeneration are of the chromophobe type, which later are transformed. Controls with simple craneotomy had only slight cellular alterations which can be attributed to the destruction of small superficial blood vessels.—J. T. L.

Anterior pituitary-like hormone: clinical study of its effects in acne vulgaris. Lawrence, C. H., J.A.M.A. **106:** 983. 1936.

A study is presented of 30 patients treated with Antuitrin-S. Three fourths of the patients were between 10 and 20 years of age and in the remaining fourth the acne had appeared during adolescence in all but 2, in both of whom it was preceded by changes in the rhythm and character of the catamenia. Ten of the patients were males, 20 were females. The menstrual periods were normal in only 6 of the 20 females. Treatment consisted of injections of 2 cc. of Antuitrin-S every other day. The duration and amount of treatment necessary to produce results varied greatly in different patients. The average dosage in the series was 3,360 R. U., the maximum 7,700 R. U., in a patient 15 years of age with severe general acne, and the minimum 300 R. U., in a patient 30 years of age with a mild eruption confined to the face and neck. In the majority of patients, improvement was apparent in from 2 to 4 weeks, and the maximal benefit was obtained in from 12 to 16 weeks. Two patients showed slight relapses, beginning 4 to 6 weeks after treatment was stopped and responding promptly to the resumption of treatment. No difference was apparent between the sexes as regards response to treatment. Ten patients were regarded as cured, since their acne had not reappeared after 2 months without treatment. Eleven were much improved, showing only an occasional papule, and 7 showed only moderate improvement.—Abst., A.M.A. Service.

Pituitary basophilism. Report of a case. Lawrence, J. H., and H. M. Zimmerman, Arch. Int. Med. **55:** 745. 1935.

The case is described of a white man, aged 44, who presented the clinical features of Cushing's syndrome of pituitary basophilism. The patient died as the result of a ruptured dissecting aortic aneurysm and cervical cellulitis. Postmortem examination revealed an inactive basophilic adenoma of the anterior pituitary gland, cortical hyperplasia or adenomas of the adrenal glands, an adenoma of one of the parathyroid bodies and diffuse skeletal demineralization.—Authors' Summary.

Experiments on the gonadotropic complex of the anterior lobe of the hypophysis. Lipschütz, A., Quart. J. Exper. Physiol. **24:** 133. 1934. Abst., Chem. Absts. **28:** 7324.

The prehypophysis of the adult female rat or guinea pig which causes estrus but no ovarian changes or luteinization in the infantile rat produces luteinization in the adult rabbit. This indicates that the luteinizing hormone is present also in the non-luteinizing hypophysis. The addition of an extract of urine of the menopause having an estrogenic but not a luteinizing action to the prehypophysis produces considerable luteinization in the infantile rat. As the prehypophysis contains the estrogenic gonadotropic factor (action in infantile rat) and the luteinizing one (action in adult rabbit) the substance which is present in extract of urine of the menopause is supposed to be a new gonadotropic hormone acting after the estrogenic one and sensitizing the follicle so as to bring about its consecutive luteinization by the luteinizing hormone.

Neutralization of the anterior hypophysis by castration in rats (Neutralisation de la préhypophyse par castration, chez le rat). Lipschütz, A., and G. Villagran, Compt. rend. Soc. de biol. **121:** 203. 1936.

The hypophyses of more than 200 adult rats, castrated and control, were tested for their luteinizing power by injection into 73 rats. Anterior lobes were dissected and weighed in 191. Castration did not change the luteinizing power in males. In females it is normally very slight but after castration it increased till it equalled that of male glands. The anterior lobes of the male are smaller than those of the female. Castration was followed by an increase in size in the male lobes so that they were as large as those of females. In both these cases, gonadectomy causes an approximation of qualities in the 2 sexes.—J. C. D.

Feminization of the male anterior hypophysis in the male rat by injections of folliculin ("Féminisation" de la préhypophyse du rat male, par injection de folliculine). Lipschütz, A., L. Palacios and J. Akel, Compt. rend. Soc. de biol. **121:** 205. 1936.

Eighty-three male rats received injections of folliculin for periods varying between 25 and 70 days. The size of the anterior lobes and their luteinizing power were then compared with those from 53 untreated animals. The glands from the treated rats resembled those from females in both respects.—J. C. D.

Specific sex differences in the anterior lobe of the human hypophysis (Différences préhypophysaires spécifiques du sexe, chez l'homme). Lipschütz, A., and M. Del-Pino, Compt. rend. Soc. de biol. **121:** 208. 1936.

Anterior lobes were obtained from 9 men, 4 pregnant women and 11 non-pregnant. The ages varied from 18 to 70. The glands were extracted and then the extracts tested for their luteinizing power on rats. The male glands were most potent, the normal female glands slightly less so and those of pregnancy practically free from effect.—J. C. D.

Disappearance of prolan from the blood of the nephrectomized rabbits (Disparition du prolan du sang de la lapine néphrectomisée). Lipschütz, A., A. Fuente-Alba and T. Vivaldi, Compt. rend. Soc. de biol. **120:** 323. 1935.

Friedman tests showed that about 30% of intravenously injected prolan disappeared, within a period of 10 hours, from the blood of previously nephrectomized rabbits. Since it has been shown that in a similar period of time 80% disappears from the blood of rabbits with intact kidneys, it was concluded that a certain amount of prolan is normally destroyed in the blood itself or in the body organs.—E. Cutuly.

Significance of the hypophysis for the antithyreotrope substance of the blood (Die Bedeutung der Hypophyse für die antithyreotrope Schutzkraft des Blutes). Loeser, A., Arch. f. exper. Path. u. Pharmakol. **180:** 458. 1936.

In order to determine if the hypophysis is the site of formation of the antithyreotrope factor, as Magistris assumes, the author investigated the antithyreotrope content of the blood serum of hypophysectomized dogs receiving the thyreotrope factor. The technique of hypophysectomy in dogs was described. The blood serum was tested on young guinea-pigs and it was found to contain antithyreotrope substance. Loeser concludes the hypophysis is not the site of elaboration of the antithyreotrope factor.—R. Cleveland.

The antithyreotrope substance of the blood and carbohydrate metabolism of the liver (Antithyreotrope Schutzsubstanz des Blutes und Kohlehydratstoffwechsel der Leber). Loeser, A., Arch. f. exper. Path. u. Pharmakol. **180:** 325. 1936.

Since it is known that the stimulation of thyroid activity by administration of the thyreotrope factor of the anterior lobe depletes the store of liver glycogen within 7-11 days, and that the simultaneous administration of the antithyreotrope factor lessens this effect, the author decided to investigate the effect of simultaneous administration of the hormone of the thyroid, thyroxin, and the antithyreotrope factor and study the effect of the liver glycogen. Guinea-pigs (49) were used in the experiment. The antithyreotrope factor in quantities as high as 16 tablets of tyronorman were unable to check the depletion of the liver glycogen, and prevent the appearance of the hyperthyroid condition of the experimental animals receiving as high as 0.8 mg. of thyroxin. The weight of the adrenals was recorded in these animals.—R. Cleveland.

The hypophysis and the mineral constituents of the blood (L'hypophyse et les substances minérales du sang). Marenzi, A. D., and R. Gerschman, Compt. rend. Soc. de biol. **118:** 488. 1935. Absts., Chem. Absts. **29:** 3385.

In dogs hypophysectomy caused a 20% decrease in plasma K and a slight decrease in Mg. Injury to the tuber caused similar but less marked changes. No significant changes in other constituents were found. In normal dogs the injection of bovine anterior hypophysis extract caused increases in plasma glucose, proteins, CO_2, P, Ca and Mg, no change in K and decreases in Na and Cl.

Brephoplastic grafts of hypophysis in the rat (La greffe bréphoplastique de l'hypophyse chez le rat). May, R. M., Compt. rend. Soc. de biol. **120:** 867. 1935.

Successful grafts of pituitaries from newborn rats were made in the anterior chamber of the eye in 2 adult male rats previously hypophysectomized. The animals became more active and gained in weight; their atrophic testes redescended into the scrotum and appeared normal. —E. Cutuly.

The effect of gonadokinetic principle of the pituitary gland and pregnancy urine on conception in the immature albino rat. McGee, W. B., New Orleans M. & S. J. **87:** 620. 1935.

White female immature rats were injected with 2 separate hormones and mated when 100 days of age. Twenty received varying amounts (6 to 90 rat units) of a pregnancy urine hormone. Sixteen received from 6 to 120 rat units of a pituitary gland hormone. The results were almost similar in both series. The 4 that received small amounts (6 rat units) had normal size litters. Two of the animals that received 90 rat units had normal size litters. Eight animals that received 30 to 120 rat units had small litters. Twenty-two animals that received 30 to 120 rat units did not conceive.—Author's Abst.

Pressor and oxytocic fractions of posterior pituitary extract. Comparative effects on blood pressure and intestinal activity. Melville, K. I., J.A.M.A. **106:** 102. 1936.

The author shows that the blood pressures and intestinal actions of pituitary (posterior lobe) extract, injected intravenously into the unanesthetized dog, vary markedly with the fraction used, even when equal pressor assayed dosages are employed. The presence of the oxytocic hormone may inhibit or abolish the typical effects of the pressor constituent. It is thus concluded that the pressor hormone alone causes under such conditions a fall of blood pressure, stimulation of intestinal activity, and defecation; while the oxytocic constituent in sufficient dosages exerts a definite antagonistic influence in respect to these actions. These observations may explain some of the conflicting reports on the clinical usefulness of the agents in question.—Abst., A.M.A. Service.

Pituitary and blood pressure in toads (Hipófisis y presión arterial en el sapo). Orias, O., Rev. Soc. argent. de biol. **10:** 91. 1934.

In Bufo arenarum the aortic pressure was measured by means of a mercury manometer. A week after simple craneotomy the average mean pressure was 40 mm. Hg.; the same figure was found after extirpation of the anterior pituitary lobe; total hypophysectomy reduced the pressure to 25 mm. Hg. This decrease was evident as early as 8 hours after operation. One month after operation the following figures were observed; craneotomy 40, anterior lobe extirpation 30, total hypophysectomy 17 mm. Hg. Implantation of either the anterior or posterior lobe reëstablished blood pressure to a normal value or even slightly higher. Lesions of the tuber cinereum caused slight variations of blood pressure in both directions. The pituitary, therefore, is a factor in the maintenance of the vascular tone in toads, the posterior lobe being of greater importance.—J. T. L.

Cardiac function of hypophysectomized toads (La función cardíaca de los sapos hipofisoprivos). Orías, O., Rev. Soc. argent. de biol. **10:** 149. 1934.

A progressive bradycardia was seen in the hypophysectomized animals, starting 3 weeks after operation, and reducing by 50% the heart rate in those that survived 6 weeks. Implantation of the anterior lobe or injection of alkaline anterior lobe extract reëstablished the normal rate. Thyroidectomy did not reduce the heart rate, but subsequent hypophysectomy was followed by the usual progressive bradycardia, which fact indicates that it is, therefore, not a consequence of thyroid atrophy produced by hypophysectomy. Anterior lobe implantation reëstablished the normal rate in thyroidectomized hypophysectomized toads. Chronaxie of heart muscle was significantly increased in hypophysectomized toads, but not in those with lesions in the tuber cinereum; implantation of anterior lobe reduced the chronaxie to normal figures. Glycogen content was considerably reduced in hypophysectomized toads but not in those with lesions of the tuber cinereum; implantation of anterior pituitary lobe reëstablished the normal value. There was no difference in the response of hypophysectomized toads with that of craneotomized controls in respect to cardiac inhibition produced by stimulation of the lunula or abdominal compression, and the impossibility of producing tetanus by faradic stimulation of the ventricles.—J. T. L.

Action of alkaline extract of anterior lobe of the hypophysis on the growth and germination of plants (Action de l'extrait alcalin du lobe antérieur de l'hypophyse sure la croissance des plantes et leur germination). Di Pascal, E. C., Compt. rend. Soc. de biol. **119:** 1127. 1935.

Unheated extracts (Evans and Simpson) were tested on various plants. The effect varied with the amount used and the kind of plant. Seeds germinated more rapidly in water alone than in the hypophyseal extract solution.—J. C. D.

Water metabolism in Bufo arenarum diuresis after total and partial hypophysectomy (Estudio sobre el metabolismo hídrico en el Bufo arenarum. La diuresis de los animales hipofisoprivos totales y privados de lobulo glandular). Pasqualini, R. Q., Rev. Soc. argent. de biol. **11:** 210. 1935.

Toads were submerged in water until completely hydrated. The diuresis was measured during 3 days, while kept in water, in the craneotomized controls it was approximately one-third of the body weight and did not vary significantly from day to day; the body weight remained constant. Hypophysectomized animals had a marked and progressive polyuria in 70% of the cases, diuresis was increased by 54.9% (average) on the 3rd day and by 92.8% on the 9th and 10th days. The body weight increased slightly 4.7% on average on the 3rd day. Removal of only the glandular lobe produced a less marked polyuria in 33% of the animals; diuresis had increased by 34.5%, 21.8%, 47.7% and 55.6% on the 2nd, 3rd, 9th and 10th day respectively; the body weight had increased by 6.6% on the 3rd day.—J. T. L.

Prolactin induces broodiness in fowl. Riddle, O., R. W. Bates and E. L. Lahr, Am. J. Physiol. **111:** 352. 1935.

Daily injections of prolactin into laying hens (20 of broody races and 9 of a non-broody race) caused all to cluck by the third to fourth day. Also, 16 from the broody races and 1 from the non-broody race nested by the fourth day. Laying ceased by the sixth day as atrophy of ovary and oviduct occurred. Only clucking was induced by prolactin in 30 non-laying hens and in 2 roosters. Gonadotropic and thyreotropic pituitary preparations, pregnant mare serum, pregnancy urine extracts, corpus luteum and female sex hormone were all unable to produce broody behavior.—R. W. Bates.

Ketonuria produced by anterior pituitary alkaline extract (Variaciones de la cetonuria por inyección de extract alcalino de lóbulo anterior de hipofisis). Rietti, C. T., Rev. Soc. argent. de biol. **10:** 136. 1934.

The extract used in these experiments was Evans and Simpson's. Injections were made intraperitoneally (7 cc. per day) (1 cc. = 0.2 gm. fresh bovine anterior lobe). In 8 normal dogs ketone bodies in urine rose from 6.2 to 22.8 mg. per kg. per day after 7 days' treatment. After subtotal pancreatectomy the rise in ketonuria was more marked—8.5 to 55 mg. per kg. per day. Thyroidectomy on the contrary considerably diminished or suppressed ketonuria provoked by the anterior pituitary. It seems therefore that ketonuria is due to the thyroid-stimulating fraction of the extract. Gonadectomy, section of the splanchnics, extirpation of the adrenal medulla and lesions of the tuber cinereum do not modify the ketonuria action of the extract. Rabbits, guinea pigs and cats also showed a rise in ketonuria after injection of the extract.—J. T. L.

PANCREAS

Observations on insulin-adrenalin treatment by the Clausen method. Andersen, T. T., Acta med. Scandinav. **86:** 361. 1935.

Seventeen diabetics were treated by adding 0.1 mg. of adrenalin to every 5.0 cc. of insulin, as recommended by Clausen. Thirteen patients were improved and 7 showed decrease or absence of glycosuria. All patients who had hypoglycemic reactions on insulin alone were entirely free from these effects on the addition of adrenalin.—J. M. L.

Insulin treatment of drug addiction. Chen, M. P., L. Y. Ch'eng, and R. S. Lyman, J. Nerv. & Ment. Dis. **83:** 281. 1936.

The rationale of this procedure is to create by means of heavy doses of insulin—20 to 30 units every 3 hours—a craving for food so intense that it takes precedence over that for the drug. Efforts are made to convert this drive for food into a drive to participate in community life via occupational therapy. In 5 morphine addicts the drug was abruptly withdrawn and insulin started. Abstinence symptoms ceased after 1 to 3 days and insulin was stopped after 3 to 5 days. One case proved uncoöperative and further treatment was refused. Two cases were dis-

charged too early to be certain about the eventual results. The remaining 2 progressed favorably.—D. E. Cameron.

The mechanism of the circulatory changes accompanying insulin hypoglycemia. Ernstene, A. C., J. E. F. Riseman, B. Stern and B. Alexander, Am. J. Physiol. **111:** 440. 1935.

Measurements of the heart rate, respiratory minute volume, oxygen consumption per minute, and cardiac minute volume output were made before and during insulin hypoglycemia in 3 groups of cats: normal animals; animals with denervated hearts; animals with denervated hearts and inactivated suprarenal glands. Control experiments were made on cats in all 3 groups. In the cats with denervated hearts and intact suprarenal glands, insulin hypoglycemia was attended by an average increase of 23% in the minute volume output of the heart and an average rise in heart rate of 34 beats per minute (26%). In contrast to this, in the animals with denervated hearts and inactivated suprarenal glands, hypoglycemia caused an average decrease of 10% in cardiac minute volume output and an average increase in heart rate of but 1 beat per minute (1%). The results indicate that both the increase in cardiac minute volume output and the rise in ventricular rate during hypoglycemia in animals with denervated hearts and intact suprarenal glands result from increased discharge of adrenin in response to stimulation of the sympathetic nerves to the suprarenal glands. The decrease in circulatory minute volume observed during hypoglycemia in cats with denervated hearts and inactivated suprarenal glands suggests that hypoglycemia per se has a deleterious effect on the myocardium.—A. C. Ernstene.

Influence of insulin on glycogen content of the frog liver (Einfluss des Insulins auf den Glykogenaushalt des Froschleber). Geiger, E., Arch. f. exper. Path. u. Pharmakol. **180:** 251. 1936.

Seasonal variations of the liver glycogen in the frog are well known; a study to determine the factors which may possibly influence this phenomenon was made on a series of 300 frogs. The frog muscles were fatigued by electrical stimulation and the liver glycogen determined following the fatigue. Insulin was administered to some of the animals, the muscles stimulated and the liver glycogen determined at the conclusion of the experimental procedure. Stimulation in the control animals led to a great decrease of the liver glycogen store; in animals treated with insulin prior of the electrical stimulation there was no decrease in the glycogen store of the liver. In normal animals the liver glycogen is lowest from March to May and is highest in November and December. In winter frogs muscle stimulation did not produce a decrease of liver glycogen. Oral administration of sugar produced an increase of liver glycogen in both winter and summer frogs; this increase disappeared within a few days in the latter animals. Insulin administration prevented this loss. The authors believed the inability of the summer frog to retain liver glycogen to be due to a relative lack of insulin in the animal's body. It was concluded that insulin reduced glycogenolysis without affecting the formation of glycogen in the liver.—R. Cleveland.

Diabetes mellitus. The differentiation into insulin-sensitive and insulin-insensitive types. Himsworth, H. P., Lancet **230:** 127. 1936.

When glucose and insulin are given simultaneously to a normal animal, the extent to which the insulin suppresses the expected hyperglycemia is determined by the sensitivity of the animal to insulin. This reaction was utilized by the author, in patients, in an attempt to differentiate between the above types. The experimental procedure followed is given. On the basis of these tests diabetics are differentiated into 2 types. (1) Those in whom injected insulin produces an immediate suppression of the expected hyperglycemia. (2) Those in whom insulin has little or no effect in suppressing this hyperglycemia. A general relationship appears to exist between onset of the disease and type of diabetes. The onset of insulin-sensitive patients is as a rule acute. The onset of insulin-insensitive patients is as a rule insidious. Those diabetics which are insulin-sensitive the cause of the disease is deficiency of insulin. In those cases which are insulin-insensitive the cause of the disease is not lack of insulin, but the restriction to a greater or less degree of the unknown sensitizing factor.—L. B. S.

One method of formation of the cells of Langerhans in the pancreas (Sur un mode de formation de cellules Langerhansiennes dans le pancréas.) Picard, D., Compt. rend. Soc. de biol. **120:** 153. 1935.

One male guinea pig which had received insulin injections and 1 pregnant female were used. These conditions cause increase in islet tissue. A certain amount of this came from

amitotic divisions of the acinar cells. Several adjoining acini may contribute cells to form a new island.—J. C. D.

New method of treatment for diabetes (Nouvelle méthode de traitement du diabète sucré). Sakharoff, G. P., and D. M. Rossiysky, Rev. franç. d'endocrinol. **13:** 470. 1935.

'Pancréotoxine' represents the serum of animals immunized against human pancreas. It was used to replace insulin and other therapy in diabetics. A course of treatment consisted of 15 to 40 injections of 0.1 to 0.2 cc. daily. Five cases were reported and others summarized. Improvement in the general state, vital activity, capacity for work, diminution or disappearance of thirst and polyuria, return to normal blood sugar, disappearance of sugar in urine and an increased sugar tolerance were noted. No untoward results are reported. Some cases remained well for 3 or 4 months following 1 months' treatment.—J. P. P.

The use of two hourly blood and urine sugar estimation in diabetes (Studien über Diabetes mellitus unter Anwendung von zweistündlichen bei Tag und Nacht entnommenen Blutzucker- und Harnproben). Vesa, A., Acta med. Scandinav., suppl. **57:** 1. 1934.

The research comprises an extensive investigation over a long period of time on 40 diabetic patients. Blood and urine examinations were started at 8 A.M. and continued bi-hourly until 8 A.M. the following morning. Blood sugar was determined by the Bang method on capillary blood. Several estimations were made on each patient on full diabetic diet (2500 calories), half diet, fasting the whole day, etc. Of the 40 patients, 15 had mild, 20 medium and 9 severe diabetes. The ages ranged from 13 to 76 years. The report includes scores of tables and charts. It appeared that the 24-hour bi-hourly blood-sugar and urine-sugar studies offered a clearer picture than the mere fasting blood-sugar and the 24-hour urine-sugar studies. The 24-hour urine-sugar estimations do not offer any more specific information than do the blood-sugar determinations although they help to orient one so long as glycosuria is present. In order to judge whether the disease is mild, medium or severe, the bi-hourly blood-sugar and urine-sugar determinations on a fasting or a diet-plus-insulin regime are of much help. In contrast the 24-hour urine-sugar determinations throw but little light on this picture.—H. J. J.

The visual mechanism in diabetes mellitis. (A comparative study of 2002 diabetics and 457 non-diabetics for control). Waite, J. H., and W. P. Beetham, New England J. Med. **212:** 367; 429. 1935.

Diabetics as a group show a higher incidence of wrinkles in Descemet's membrane, deep retinal hemorrhages, depigmentation of iris epithelium, transitory refractive changes, and toxic amblyopia, than do non-diabetics. There is an equal incidence of cataracts of all sorts except flocculi in juvenile diabetics, of iritis, of atrophy of the optic nerve, and of arcus. Paresis of accommodation was demonstrated in 21% but it was a transitory condition improving with diabetic therapy. Glycogen infiltration of ocular tissues was uniformly found. Transitory refractive changes were found in 6%. Slowly progressive, bilateral cataracts in juvenile diabetics were found 11 times in 297 patients. Vitreous hemorrhage, associated with proliferation of connective tissue and blood vessels in retina and vitreous appeared in 31 patients, in whom the dominant findings were cardio-renal-vascular disease and disease of the retinal venous tree. The prognosis for sight is poor in spite of the best diabetic handling. Deep retinal hemorrhages in diabetics multiply with age, and multiply with continued duration of diabetes out of proportion to the age factor, but they show no obvious correlation with sclerosis of retinal vessels, with vascular hypertension, with renal disorders, with insulin dosage, or with blood sugar or calcium levels.—J. C. D.

Neuritic manifestations in diabetes mellitus. Jordan, W. R., Arch. Int. Med. **57:** 307. 1936.

In a study of 226 cases of diabetes in association with neuritic manifestations, the author noted manifestations of 3 types: hyperglycemic, degenerative and neuritic. The 34 cases of hyperglycemic type were characterized by neuritic symptoms. The diabetes was uncontrolled at the onset of the symptoms in each case. Hyperglycemia and glycosuria were the only detected diabetic factors present. Regulation of the diabetes gave relief within a few days. A more serious neuropathy developed subsequently in 4 cases. Forty-five cases of diabetes with relatively mild chronic neuropathy were observed. To these were added 27 cases considered separately as of the circulatory type at first, because of markedly sluggish circulation in the legs. There were evidences, however, of chronic lesions elsewhere than in the legs, and in etiology, manifestations

and prognosis, the condition seemed to be of the same type as that in the original 45 cases. Hence the groups were combined. The outstanding symptoms were pain, cramps and paresthesia, of greatest intensity at night, hyporeflexia and areflexia, muscular paresis, tenderness of nerves and muscles, hypesthesia, abnormal pupillary reactions and paresis of the muscles of the bladder. The legs were involved more frequently than any other part of the body. Although usually progressive over a long period of time, the neuropathy occasionally improved considerably. The condition occurred in patients 40 years old or more, and arteriosclerosis was present in every case. As judged by sluggishness or absence of tendon reflexes of the legs, this type of neuropathy involves over 40 percent of all diabetic patients. The 120 cases of diabetes with relatively acute and usually severe neuropathy represented the cases of diabetic neuritis and diabetic tabes. Among 1,000 consecutive cases of diabetes observed during a period of less than two and one-half years, there were 25 cases of neuritis. The neuropathy involved the legs in over 65 percent of the cases. Pain, paresthesia, hyporeflexia and areflexia, muscular paresis, tenderness of the nerves and muscles, hypesthesia and hyperesthesia were the most frequent manifestations. Evidence of vascular disease, primarily arteriosclerosis, was present in 95.8 percent of the 120 cases.—I. B.

Neuropathy in diabetes. Lipid constituents of the nerves correlated with the clinical data. Jordan, W. R., and L. O. Randall, Arch. Int. Med. **57:** 414. 1936.

The authors made analyses of various lipid constituents of 52 nerves from persons with diabetes and of 23 nerves from persons without diabetes. The average phospholipid, cholesterol and cerebroside content of the nerves from diabetic patients was considerably lower than that of the nerves used for controls. The nerves from the lower part of the legs of diabetic patients showed much greater damage than nerves from the pelvic level. This was not true in the one nerve from the lower part of the leg of a control subject as compared with the pelvic nerves from control subjects. The greater the vascular disease in a diabetic patient the greater the damage to the nerves. In 2 patients without diabetes who had arteriosclerosis this pathologic change was not found, indicating that vascular disease is not the sole cause of neuropathy in diabetic patients. Previous inadequate control of the diabetes seems to affect the nerves adversely to a slight extent. The severity and duration of the diabetes seemed not to affect the nerves, or else the effect was nullified by other factors. The chemical pathologic changes in the nerves of diabetic patients seemed to exceed or to precede the clinical manifestations of neuropathy.—I. B.

Therapeutic applications of insulin. Joslin, E. P., H. F. Root, Priscilla White and A. Marble, J.A.M.A. **105:** 359. 1935.

Ordinarily a dose of insulin exerts an action for 8 hours. In infections injections of insulin are given every 6, 4, 3 or 2 hours, and in coma it is often injected every 30 minutes. Insulin lowers the percentage of sugar in the blood and favors its storage and utilization in the liver, muscles and skin in the form of glycogen. When the blood sugar drops in insulin shock the counter-regulatory mechanism of the body, through stimulation of the sympathetic system, tends to relieve the resultant hypoglymecia by favoring the discharge of glycogen from the liver. If this counter-regulation goes too far and the liver glycogen is depleted unduly, the liver acts less well in furthering carbohydrate metabolism. This state of an approaching depletion of glycogen in the liver can be forestalled by the administration of carbohydrate, and if done when the blood sugar is already low and falling, it is well tolerated by patients. In general 1 unit of insulin will metabolize 1 or 2 gm. of carbohydrate, and perhaps even from 3 to 6 gm. The maximum effect of an injection of insulin occurs in an hour. The blood sugar is higher before and rises higher after breakfast than before and after the other meals; hence insulin is given at a longer period before breakfast. Contraindications to insulin treatment are anaphylaxis, insulin lumps or tumefactions and insulin atrophies. Insulin atrophies are far more annoying, more disfiguring and more permanent than insulin anaphylactic phenomena or insulin lumps. They occur as a rule at the exact site or near the site at which insulin has been administered. The cause of these atrophies is not known. Symptoms of an overdose of insulin are identical with those resulting from a fall in the percentage of sugar in the blood below normal. Dilated pupils, firm eyeballs, diplopia, pallor, normal respiration and blood pressure, and normal or sometimes rapid pulse are symptoms of insulin overdosage and may be followed by complete unconsciousness and convulsions. The latter state may last for hours and infrequently end in death. Treatment of the insulin reaction is simple; 5 or 10 gm. of dextrose orally usually relieves the patient in a few minutes. Occasionally 3 times as much is required.

Delayed absorption of food is a menace to an insulin treated diabetic patient. Exercise tends to lower the blood sugar in the diabetic patient in whose body there is an adequate supply of insulin. Thus exercise along with diet and insulin is now accorded a definite and prominent place in the everyday treatment of diabetes. In the very young and in the very old, insulin should be begun with small but frequent doses. An unfounded prejudice exists against the use of insulin in heart disease. Insulin has a useful effect on the carbohydrate metabolism in nondiabetic persons. In cases of hyperinsulinism the symptoms of hunger, nervousness, sweating, fainting, epileptiform convulsions and unconsciousness are due to the hypoglycemia and are usually relieved by food or by dextrose given intravenously.—Abst., A.M.A. Service.

PARATHYROIDS

Fluorine as specific for parathyroids (Das Element Fluor als Spezifikum für die Nebenschilddrüse). Callam, M., München. med. Wchnschr. **82:** 1534. 1935. Abst. J.A.M.A. **105:** 1563.

The author points out that fluorine can be used with good results in disorders in which there is insufficiency of the parathyroids. He prescribes a 1% solution of chemically pure sodium fluoride in distilled water, of which the patient takes from 5 to 8 drops in a teaspoonful of water 4 times daily. He asserts that, if the sodium fluoride is given in these quantities, there are no toxic effects. An indication of the efficacy of sodium fluoride is the disappearance of the cicatricial keloids after from 2 to 3 months of medication. Callous scars decrease in size and become so soft that they hardly differ from the surrounding skin. This applies to external scars and is true also of urethral, vaginal, esophageal or other scars. The author thinks that, aside from surgical stimulation of the parathyroids, the medication with sodium fluoride is the best treatment for scars. That sodium fluoride has connections with the parathyroids is indicated by the author's observation that, in the dosage mentioned, sodium fluoride is a good antispasmodic in infantile eclampsia and in epilepsy of children. Moreover, sodium fluoride is helpful in suppurations and inflammations of the bones, in exostoses and in fractures that fail to heal. The author thinks that it might likewise be tried in bone sarcoma. He suggests investigations on the effect of fluorine on the calcium content of the blood, osteomalacia, osteitis, fibrosa and experimental tetany following removal of the parathyroids.

The mechanism of parathyroid hormone action. Collip, J. B., L. I. Pugsley, H. Selye, D. L. Thomson, Brit. J. Exp. Path. **15:** 335. 1934. Abst., Chem. Absts. **29:** 3013.

The characteristic effect of the parathyroid on the bones of rats is obtainable after bilateral nephrectomy, and hence cannot be ascribed to an action of the hormone on the renal threshold for phosphate. Since the histological changes in the bones in hypervitaminosis D are entirely different from those observed in experimental hyperparathyroidism, it is difficult to believe that irradiated ergosterol acts merely by stimulating production of the parathyroid hormone.

Action of parathyroid extract on carbohydrate metabolism (Paratiroidi e ricambio degli idrati di carbonio; l'azione dell'estratto paratiroideo sul ricambio degli idrati di carbonio nei soggetti normali). Ferrannini, A., Policlinico **42:** 285. 1935. Abst., J.A.M.A. **104:** 2399.

The author studied the action of parathyroid extract on glycemia of normal persons. He carried out his investigations in 3 groups of individuals: In one group he investigated the action of parathyroid extract on glycemia, in another its action on sugar tolerance and in the third the relation that exists between the action of parathyroid extract and that of epinephrine on glycemia. From the results of his investigations the author concludes that parathyroid extract produces a decrease of glycemia, which oscillates between 13 and 56%. These results were seen in almost all cases (13 of 14). Parathyroid extract produces also an increase in the tolerance to carbohydrates. Its action on glycemia is similar to that of insulin.

Hypocalcemia of pancreatectomized dogs (Hypocalcémie des chiens sans pancréas). Gerschman, R. and A. D. Marenzi, Compt. rend. Soc. de biol. **120:** 737. 1935.

Ablation of the pancreas in 40 dogs produced a decrease in blood calcium and a rise in plasma phosphates. These changes were attributed to a condition of hypoparathyroidism resulting from lesions in the parathyroids consequent to pancreatectomy.—E. Cutuly.

The action of parathormone. Goadby, H. K., and R. S. Stacey, Biochem. J. **28:** 2092. 1934. Abst., Chem. Absts. **29:** 2587.

The first apparent action of parathormone is to increase the rate of excretion of P. This increased excretion is independent of the concentration of inorganic P in the blood plasma. The point of action of parathormone is limited to 3 possibilities, the kidney inorganic phosphate excretion mechanism, the kidney phosphatase or the inorganic P reserves in the blood or tissues.

Serum calcium, inorganic phosphorus and phosphatase activity in hyperparathyroidism, Paget's disease, multiple myeloma and neoplastic disease of the bones. Gutman, A. B., T. L. Tyson and Ethel B. Gutman, Arch. Int. Med. **57:** 379. 1936.

This study is a review and appraisal of the available data relating to hyperparathyroidism, Paget's disease, multiple myeloma and neoplastic diseases of the bones, with additional observations on personally studied cases. Of the cases of hyperparathyroidism in which determinations are recorded in the literature, about four-fifths showed consistent hypercalcemia, with a calcium content of more than 12 mg. per 100 cc., and about one-half, consistent hypophosphatemia, with an inorganic phosphorus content of less than 2.5 mg. Increase in blood phosphatase activity was found in every case of classic hyperparathyroidism with definite changes in the bones in which determinations were made (28 cases). Complicating factors affecting these values and their significance in differential diagnosis are discussed. The question of 'secondary' hyperparathyroidism is also considered.—I. B.

Alterations in the parathyroids of pancreatectomized dogs (Altérations des parathyroïdes des chiens pancréatoprives). Houssay, B. A. and R. Sammartino, Compt. rend. Soc. de biol. **120:** 735. 1935.

The major histological changes occurring in the parathyroid glands of 29 pancreatectomized dogs included vacuolization, liquefaction and disintegration of the cellular protoplasm.—E. Cutuly.

Relation of parathyroids to the insulin mechanism (Das Verhältnis der Epithelkörperchen zum Inselapparat. [Ein kasuistisch-experimenteller Beitrag]). Mann, F., Endokrinologie **14:** 153. 1934. Abst., Chem. Absts. **28:** 5865.

Description of a clinical case, in which a latent parathyroid hypofunction resulting from an old Pb injury was associated with a severe epileptoid reaction and psychic disturbances in response to insulin. No change in rheobase or chronaxie of the muscles is found in normal individuals under insulin or with high blood sugar, nor in 2 post-operative cases with latent tetany.

Effect of parathyroid extract on the glycemia in diabetics (Action de l'extrait parathyroïdien sur la glycémie des diabétiques). Olmer, J., J. E. Paillas and B. Sicnasi, Compt. rend. Soc. de biol. **119:** 1264. 1935.

In 13 human diabetics, 5 males and 8 females, parathyroid extract injected intravenously lowered the blood sugar level.—J. C. D.

Histologic study of parathyroids in chronic rheumatic polyarthritis and spondylo-arthritis. Shkurov, B. I. and K. F. Elenevskiy, Ortop. i. travmatol. **6:** 17. 1934. Abst., J.A.M.A. **105:** 325.

The authors examined 91 histologic preparations of parathyroid tissues removed for therapeutic purposes from 77 patients, 30 of whom were suffering from chronic rheumatic polyarthritis and 47 from spondylo-arthritis. The presence of parathyroid tissue in the removed material was established in each instance by histologic examination. The size of the glands removed did not exceed that given by most authors for the normal gland. On the basis of their observations the authors suggest that the secretory reaction is in all probability not limited to the chief cells and that oxyphil cells likewise have a secretory function. They found oxyphil cells singly and in islets in 53 preparations, cells representing a transitional stage from the chief to the oxyphil type in 34 and a colloid-like substance in 32. Clinically the cases presented increased blood calcium content, stiffness and ankylosis of the joints. The preoperative blood calcium was above normal in 22 (up to 16 mg.), normal in 11 and below normal in only 2.

The 22 patients with hypercalcemia showed, after parathyroidectomy, an average diminution of 3 mg. per 100 cc. of blood serum. The clinical results were gratifying. Of the 81 patients submitted to partial parathyroidectomy, only 3 did not show any improvement, and in 2 the progress of the morbid condition was not arrested. The authors conclude that the oxyphil cells signify an increased function of the parathyroid and that chronic rheumatic polyarthritis and spondylo-arthritis are caused by hyperactivity of the parathyroid glands.

THYROID

Cachexia hypophyseopriva (Simmond's disease). Report of a case in an adolescent girl. Aldrich, C. A. and J. A. Walsh., J. Pediat. **7:** 490. 1935.

A 15 year old girl presented the classical symptoms of loss of weight (30 lb.), cachexia, anorexia, cessation of menses, low basal metabolic rate and splanchnomicria. There was an improvement on anterior pituitary extract but not until thyroid extract was discontinued. —M. B. G.

Functional significance of the interstitial islets in the thyroid of the guinea pig (Valeur fonctionelle des îlots interstitiels de la thyroïde du cobaye). Bastenie, P. and G. Roost, Compt. rend. Soc. de biol. **120:** 512. 1935.

Nine female guinea pigs were injected intraperitoneally with thyreotropic hormone for 3 to 5 days. The basal metabolism of 2 of these animals increased 30 to 40% by the fifth day. Histologically the thyroids of this group showed diminution of colloid, cylindrical transformation of vesicular cells and numerous mitoses. Compact islet tissue was not found in appreciable amounts. In a second group of 7 females thyreotropic hormone was administered over a period of 40 days. The basal metabolism of 5 of these animals, following a slight initial rise, progressively decreased to sub-normal values. Histologically the thyroid glands of these animals showed transformation of vesicular tissue into compact masses of islet interstitial tissue. It was concluded that the occurrence of such islet tissue indicates a reduced endocrine activity of the thyroid.—E. Cutuly.

Glutathione of red blood cells in hyper and hypothyroidism (Glutation eritrocitario en el hiper e hipotiroidismo). Beaux, A. R., Rev. Soc. argent. de biol. **11:** 489. 1935.

Thyroidectomy in dogs was followed by an increase in glutathione of red blood cells; experimental hyperthyroidism on the contrary produced a decrease. Thyroidectomized dogs showed a decrease to normal in blood glutathione when treated with thyroid. Hyperthyroid dogs had an increase in blood gluthathione after thyroidectomy. In patients with hyperthyroidism the blood glutathione was low but it increased gradually after thyroidectomy.—J. T. L.

Dangers in the treatment of exophthalmic goiter by di-iodotyrosine (Nadeelen van dyoodtyrosine by de behandeling der hyperthyreoidie). Beekhuis, W. H., Nederl. tijdschr. v. geneesk. **79:** 3963. 1935.

According to Plummer exophthalmic goiter in the Netherlands is mostly treated by operation after previous use of iodine. Trials with di-iodotyrosine without surgical intervention did not give good results. Only in very mild cases may it be used, under control of the basal metabolic rate. In more severe cases it may be used only as a preparatory measure before operation. It is not more efficient than other preparations of iodine and its effect, as in iodine, is only of short duration. On suspending the use of di-iodotyrosine, often an exacerbation is seen of all the symptoms. Three cases illustrate this conception.—L. M.

The follicular hormone inhibits the function of the thyroid (L'ormone follicolare inibisce la funzionalita' Tiroedea). Benazzi, M., Boll. soc. ital. biol. sper. **8:** 790. 1933. Abst., Chem. Absts. **29:** 2588.

The administration of folliculin in marked doses slows the functional rhythm of the mouse thyroid almost to the point of causing a complete inhibition of the processes of resorption of the colloid substance. The disappearance or a marked diminution of the chromophobe vacuoles may also be noted. This is followed by an accumulation of eosinophile colloid in the lumens

of the folliculi. The parietal epithelium appears less developed in proportion to the slowing down or arrest of the secretory activity.

Total thyroidectomy for intractable heart disease: Summary of two and one-half years' surgical experience. Berlin, D. D., J.A.M.A. **105:** 1104. 1935.

An evaluation of the clinical results that the author obtained in those patients, who were operated on from 1 to 2½ years ago reveal that of 36 patients with angina pectoris 50% have been markedly improved. In this category are included patients who were previously incapacitated and were sufficiently relieved by operation so that they no longer required medication and were able to return to work. In an additional 17% the attacks of coronary pain were diminished in frequency and severity. The improvement in this group is classified as moderate. The results in patients with congestive failure are equally satisfactory. Approximately 70% derived a degree of improvement which was greater than that obtained by adequate medical therapy prior to thyroidectomy. Of these totally incapacitated cardiac patients 38% were markedly benefited by operation to the extent that they were able to undertake, without circulatory embarrassment, activity such as had been previously denied them. An additional 31% showed a moderate increase in their capacity for work without recurrence of failure. Approximately 30% of the patients suffering from either angina pectoris or congestive heart failure showed little or no improvement following operation. The lack of improvement in this group can be attributed in most instances either to a rapid progression of the underlying disease or to an unduly low preoperative basal metabolic rate. The results of this group emphasize the need of utmost care and caution in the selection of patients for operation.—Abst., A.M.A. Service.

The incidence of goiter amongst Saskatoon school children. Binning, G., Canad. M.A.J. **32:** 533. 1935.

School children (5808) in Saskatoon were examined for goiter, and 718 cases found. The incidence in girls was nearly double that in boys, and the goiters tended to be larger. Goiter began to be prevalent at 9 years. The incidence rose at 11 and 15 years, owing to an increase of the moderate-sized goiters. Racial origin seemed to be the factor of greatest importance in the incidence of goiter. This was not due to the diet or water supply, because at least half of the races in whom goiter was most frequent were on the same diet as those of British origin. The higher incidence of goiter, the lower one went in the social scale, seemed to be due to the presence of a greater number of those races prone to goiter in the lower social classes. The larger percentage of Austrians, Ukrainians, Poles, Russians, Dutch and Germans 'on relief' than the average was discovered in the course of survey.—Author's Summary.

Pressoreceptor nervous system and its practical significance in surgery (Das pressorezeptorische Nervensystem und seine praktische Bedentung in der Chirurgie). Braeucker, W., Beitr. z. klin. Chir. **158:** 309. 1933. Abst., J.A.M.A. **101:** 1596.

The concept of a pressoreceptor nervous system consisting of 4 nerves, the 2 superior cardiac nerves and the 2 carotid nerves, branches of the glossopharyngeus, is incomplete. The system in rabbits is represented anatomically by a very complex network of periarterial plexuses which spread from the cardiac plexus over the arch of the aorta, the common carotid artery, its bifurcation and its branches. The afferent pressoreceptor paths are not limited to the 4 nerves as previously considered but are a part of the peripheral vegetative nervous system of the neck and the upper part of the thorax. The same anatomic relations were found in man except that the pressoreceptor nervous system extends to the thyroid gland and forms a plexus within the so-called thyroid capsule. Central stimulation of sectioned ends of pressoreceptor nerves causes a two-fold stimulating effect on the medullary centers—vasodilation and slowing of the heart. The question of the possible reflex effect of trauma to the nerve plexus of the thyroid capsule in the course of delivery and resection of the gland presents itself. The author found that signs of bulbar paralysis developing in animals in the course of dissection of the neck could be successfully combated by a timely venesection. He advances the theory that sudden death in the course of a thyroidectomy, and the so-called postoperative thyrotoxic crises, are reflex phenomena resulting from irritation of pressoreceptor nerves. He reports the histories of 5 patients with thyrotoxicosis, who developed in the course of a thyroidectomy grave signs of impending paralysis of the bulbar centers. Prompt venesection, with withdrawal of from 200 to 300 cc. of blood from the anterior jugular vein, gave immediate relief of these symptoms.

Total thyroidectomy in the treatment of patients with congestive heart failure and angina pectoris, with note on anaesthetic. Brenner, O., H. Donovan, and B. L. S. Burtagh, Brit. M. J. **2:** 624. 1934.

The whole thyroid gland was removed in 6 patients without hyperthyroidism, showing incapacitating congestive heart failure, angina pectoris, or both, in whom all previous treatment had been ineffectual. There were no operative deaths, though 1 patient with a very high blood pressure died of cerebral haemorrhage 6 weeks after the operation. All 6 patients showed slight to fairly marked improvement in their circulatory symptoms after the operation, and were capable of more exertion. One of them, who had done no housework for over 6 months, is now doing all her housework except the heavy washing. The cases are all too recent for the permanent effects to be judged, but it is hoped to report on their condition again in about 12 months' time. Slight signs of hypothyroidism appeared in 2 cases only. The rationale of this method of treatment is discussed.—Author's Summary.

The iodine relationships of thyroid disease. Curtis, G. M., Surg., Gynec. & Obst. **62:** 365. 1936.

The micromethod of Davis and Phillips was utilized in more than 6000 iodine determinations to establish the iodine content of the thyroid gland, iodine of the blood, and loss of iodine from the urine. The normal human thyroid gland contains 10 mg. of iodine at a concentration of 40 mg.%, wet weight. This is found principally in the colloid. The geographic and pathologic variations are discussed. The normal iodine content of the human blood is 0.012 mg.%. Thus the entire iodine content of the human blood stream is less than 1 mg. The blood iodine is affected by diseases of the thyroid gland. In the majority of patients with other than thyroid diseases there is a normal blood iodine. The pathologic conditions bringing about changes in the blood iodine content is discussed. The administration of thyroxine, which is 65% iodine to patients with hypothyroidism is followed by a prompt increase in the blood iodine. The daily loss of iodine in the urine is variable in normal individuals kept on a constant monotonous diet. The average loss being from 31 to 53 micrograms. There is a periodic increase of iodine excretion in women during the course of the complete menstrual cycle. One woman lost 42 to 53 micrograms daily just previous to the onset of menstruation, 108 to 112 during menstruation, and 41 to 65 during the days immediately following. The blood iodine rose to 43 on one of the days of increased iodine loss. These cyclic variations of iodine loss in the urine appear to be associated with the periodicity of the ovarian activity. An iodine diabetes was demonstrated in 5 hyperthyroid patients ages 27 to 73. The average basal metabolism was plus 43. The average daily loss in the urine 139 to 143 averaging 304 micrograms, which is approximately 6 times more than the normal iodine excretion in the urine.—L. B. S.

Control of thyroid activity with animal blood (Die Hemmung der Schilddrüsent ätigkeit durch Tierblut). Eitel, H., and A. Loesser, Klin. Wchnschr. **13:** 1742. 1934.

The authors favor the use of animal blood in the treatment of Basedow's disease and believe the results to be due to the existence of an anti-thyroid substance in the blood employed. It is suggested that the use of commercial preparations of animal blood serum in the treatment of Basedow's disease is justified.—I. B.

Effect of vitamins A, C, D on normal and hyperactive thyroids; inhibiting action of vitamins A and C on experimental hyperthyroidism (Action des vitamines A, C, D sur la thyroïde normale et sur la thyroïde hyperactivée par la thyréostimuline préhypophysaire; effet inhibiteur des vitamines A et de l'acide ascorbique dans l'hyperthyréose expérimentale). Elmer, A. W., B. Giedosz and M. Scheps, Compt. rend. Soc. de biol. **120:** 560. 1935.

In the dosages employed vitamins A, C, and D had no effect on the histological structure of the thyroids of normal guinea pigs. Vitamins A and C under certain conditions prevented the thyroid hyperactivity produced by the thyrotropic hormone.—E. Cutuly.

The phenomena of anuran metamorphosis. The development of the thyroid gland. Etkin, W., J. Morphol. **59:** 69. 1936.

This paper presents a study of the growth and histological development of the thyroid in 3 species of frogs, the small Pseudacris triseriata, the large Rana catesbeiana and the intermediate Rana palustris. It was found that the history of the gland in relation to metamorphosis

is essentially the same in all. Previous to metamorphosis the gland is small, and the cells very poor in cytoplasm. At the beginning of metamorphosis (*i.e.,* rapid hind leg growth) the thyroid shows a marked acceleration of growth and activity, which continues and reaches a peak at the time of the emergence of the front legs. Late in metamorphosis a regression occurs. The conclusion is drawn that there is a rising concentration of thyroid hormone during the course of metamorphosis. The author has previously shown that such a rising concentration will induce the normal pattern as to sequence and time spacing of metamorphic events. The beginning of metamorphosis is conceived as dependent upon the time of stimulation of the thyroid and the pattern of its subsequent continuance as dependent upon the pattern of response of this gland.—Author's Abst., Wistar Bib. Service.

The iodine content of the thyroid in relation to alterations in organs of the genital system in sterile cattle (Ricerche sul contenuto in iodio delle tiroidi in rapporto alle alterazioni degli organi della sfera genitale nelle bovine sterili). Falaschini, A., and E. Vallesi, Biochem. e. terap. sper. **21:** 297. 1934. Abst., Chem. Absts. **29:** 502.

The relative I contents of the thyroid in pregnancy are normal or slightly reduced; they are normal in cows rendered sterile by lesions of the genitals. As the thyroid is enlarged in both cases, a higher total I value is found.

Effect of thyroid-stimulating hormone on the histological structure of endocrine glands (Influence de la thyréostimuline sur l'aspect histologique des glandes endocrines). Giedosz, B., Compt. rend. Soc. de biol. **120:** 555. 1935.

In guinea pigs intraperitoneal injections of thyrotropic hormone for 5-6 days caused, in addition to the recognized changes which occur in the thyroid, increase of basophiles in the anterior hypophysis; absence of follicles in the ovaries; hyperemia of the medulla, and hyperemia and increase in size of the cortex of the adrenals. The ovaries and adrenals were probably influenced indirectly through the anterior pituitary.—E. Cutuly.

The effect of thyroxin on temperature regulation (Uber die Beeinflussung der Temperatur-Regulierung durch Thyroxin). Glaubach, S. and E. P. Pick, Arch. f. exper. Path. u. Pharmakol. **179:** 678. 1935.

The effect of thyroxin on temperature regulation was made on male guinea-pigs with spinal cords transected at the level of the 5th-8th thoracic vertebra. Novocain, 20 mg. per 100 gm. of animal, produced consistently a drop in temperature in the normal pig and in the animals with transected cords. The effects of thyroxin on this lowering of temperature by the novocaine hydrochloride was studied in the normal and operated animals. The subcutaneously administered novocain produced a lower and more prolonged drop of body temperature in the operated animal than in the normal. Thyroxin administered at the same time prevented any temperature drop in the normals as a result of novocain injection, while in the operated animals the thyroxin could but partially compensate the action of the novocain. Following removal of the haunches and leg musculature after section of the femoral and ischial nerves in the operated animals, the temperature level was no lower than that of the normal animals; thyroxin administered to such animals had the same effect as in the normal animals.—R. Cleveland.

Method for determination of small quantities of fluorine in human organic fluids (Método para dosar las pequeñas cantidades de fluor de los líquidos orgánicos humanos [humores, secreciones y excreciones] y los resultados obtenidos endiversos estados patológicos). Goldemberg, L., and J. Schraiber, Rev. Soc. argent. de biol. **11:** 525. 1935.

The concentration of fluorine was found to be 0.05-0.08 mg.% in human serum. It was absent in human and bovine red cells which have a membrane that is not permeable to this element. The C.S.F., urine, milk, feces, bile and pleural effusion had the same Fl concentration as the blood serum. An inverse ratio of Fl and basal metabolism and blood iodine was found. In no disease, outside of thyroid disorders, was there a significant variation of the serum Fl. Injection of Fl produced a transitory increase in plasma with return to normal in 24 hours. It was eliminated by the kidney almost exclusively, little or none was found in the feces and saliva. Sperm was found to be very rich in Fl, which was increased in cases of gonococcic urethritis and orchitis. Normal human adults eliminated from 0.9 to 1.1 mg. Fl, 80% by the kidney, in 24 hours when on the usual mixed diet.—J. T. L.

Clinical hyperthyroidism associated with a normal metabolic rate. Gordon, S., and R. R. Graham, J. Nerv. & Ment. Dis. **82:** 201. 1935.

Seventy-one cases diagnosed as clinical hyperthyroidism with normal metabolic rates are presented. The normal rate is taken as from —10 to +15. Seventeen had diffusely nodular glands; 54 nodular. The average time since operation was 3.7 years. No definite clinical syndrome was established. Fatigue, irritability, nervousness, palpitation, excessive perspiration, headache, restlessness and sleeplessness were the main symptoms. There was no significant weight change. Relief of symptoms occurred in a considerable proportion of the cases by operations upon the thyroid. The weight often improved. It was felt on the basis of results of treatment of this group that there was sufficient justification to establish the disease as a clinical entity, particularly in patients with nodular goiter. In no patient should a diagnosis of clinical hyperthyroidism be made in the presence of a normal basal metabolic rate unless a very careful and thorough investigation fails to reveal any other organic or psychological basis for the patient's ill health.—Author's Abst. (Abbreviated)

The influence of thyroxin on oxygen consumption of living tissue (Über den Einfluss von Thyroxin auf den Sauerstoffverbrauch uberlebender Gewebe). Haarmann, W., Arch. f. exper. Path. u. Pharmakol. **180:** 167. 1936.

The author investigated the effect of thyroxin in various concentrations on the oxygen consumption of tissues, such as the pectoral and leg muscles, liver, heart, kidney, and brain in the cat, rabbit, dog, rat, frog, chicken, pigeon, etc. The determinations were made by means of the Warburg apparatus and the Barcroft manometer. Finely pulverized tissue, 0.5 gm., suspended in a phosphate buffer solution at a pH of 7.36 was used in each determination. Precautions against anerobic processes were taken in order to secure accurate results. The temperature was maintained at 30° C. and the values reported were the averages of duplicate determinations interpreted in terms of the amount of oxygen consumed by 1 g. of tissue in 1 hour. It was found that concentrations of thyroxin between 10^{-10} and 10^{-13} increased the oxygen consumption of the tissue, and that higher concentrations inhibited it. The maximal increase was between 10^{-11} and 10^{-16}. In the living body failure of thyroxin production and the massive overproduction of it give similar results. Within these two extremes normal thyroid function and the clinical manifestations of hyperthyroidism occur.—R. Cleveland.

Development of the thyroid gland in the chick embryo. Hopkins, Marie L., J. Morphol. **58:** 585. 1935.

The developing chick thyroid consists of epithelial plates or cords suspended in a sinus, probably lymphoid. These cords in cross section have the appearance of follicles. Growth in size is accomplished by fusion of follicles. Growth in number of the latter by proliferation of inter-follicular epithelial cells. Colloid first appears on the 10th day of incubation. The tadpole test indicates the beginning of functional activity at this time. During development there is a progressive decrease in the cytoplasm-nucleus ratio from 3.3: 1 on the third to 1.5: 1 on the 19th day, as measured in area, not volume. Not only is chromophobe and chromophile colloid present in different cells of the same follicle at the same time, but both are occasionally present in the same cell at the same time. Evidence of heightened activity in the chick thyroid are as Uhlenhuth described them in salamanders: namely, high columnar cells, apically located secretion granules, and vacuoles of chromophobe colloid. Follicles in late developmental stages have, at some time during their functional cycles, tubular exits, which are thought to represent one path through which colloid reaches the circulatory spaces. Vital staining indicates that elements of the reticulo-endothelial system are present in the developing thyroid. Phagocytic cells in the gland of one embryo were observed phagocytosing entire erythrocytes.—Author's Abst., Wistar Bib. Service.

Diabetes and hyperthyroidism. Joslin, E. P., and F. H. Lahey, Ann. Surg. **100:** 629. 1934. Abst., Chem. Absts. **29:** 504.

Glucosuria is so related to hyperthyroidism that it must not be confused for diabetes in cases of the former. The former disease intensifies the latter, increasing the demands for insulin. After surgical relief of hyperthyroidism there is an increase in carbohydrate tolerance of 30 grams or more and a marked diminution in the need for insulin.

Psychoses of myxedema. Karnosh, L. J., and R. E. Stout, Am. J. Psychiat. **91:** 1263. 1935.

A woman developed a retarded and depressed affectivity, poorly systematized ideas of persecution and vague hallucinatory experiences accompanied by the physical and metabolic

signs of myxedema. Improvement in the psychotic manifestations following the administration of desiccated thyroid was striking but incomplete. A relapse of her mental and physical symptoms promptly followed the halting of glandular treatment; the mental recovery following the resumption of treatment exceeded the original improvement. A 58-year-old woman with moderate arteriosclerotic changes, who had always been reserved and sensitive, when subjected to the metabolic sluggishness of a profound myxedema became confused, affectively retarded, hallucinated and depressed. A 35-year-old, extremely schizoid single woman, with poor intellectual and affective qualifications under the added handicap of insufficient thyroxin, underwent a complete introversion and affective distortion. The physical manifestations of hypothyroidism were relieved by adequate treatment, but no mental improvement occurred. A young Jewish girl just entering the menarche developed a rabid hyperthyroidism that was treated by thyroidectomy after which myxedema supervened. She became seclusive, apathetic, incoherent in speech and depressed. For a short period she was very irrational, then more erratic mentally, extremely psychasthenic and negativistic. Under thyroid and prolan medication gradual improvement ensued. It appears that in elderly patients with general and cerebral arteriosclerosis, myxedema leads to a further reduction of the cerebral circulation, and thus to an organic psychosis characterized by a confusion, loss of memory, vague hallucinatory experiences and a loosely constructed paranoid delusional system, accompanied by mild depression and retardation. Thyroid medication may lead to striking relief of all symptoms. Young individuals with myxedema, especially those with an excessively schizoid personality, are prone to develop a vivid dissociation and hebephrenic regression. If well developed, these changes may not be reversible, even with adequate treatment.—R. G. H.

Ultimobranchial body and thyroid gland in the fetal calf. Kingsbury, B. F., Am. J. Anat. **56:** 445. 1935.

Four pharyngeal pouches are developed in the calf, the fourth seemingly not reaching the ectoderm. The ultimobranchial body arises from the fourth pharyngeal outpocketing as an undeveloped fifth pouch. Its boundaries as a separate outpocketing later become lost. For the caudal outpocketing of the pharynx (fourth pouch plus ultimobranchial body) the term caudal pharyngeal complex is employed. The complex differentiates a parathyroid (IV) and frequently a thymus (IV), but these retain a continuity with the rest of the complex. The caudal portion of the complex (ultimobranchial body) becomes fused with the expanding thyroid. The chromatic character of the complex is marked throughout its history. This enables its ready differentiation from thyroid in successive stages of its incorporation. The growth of mesenchyme (connective tissue) sets off distinctly the body of the complex (and its stalk) from the surrounding thyroid. The growth of the thyroid deeply buries the fundus of the complex, and its cords. A vasculo-stromal hilus of the lateral thyroid lobe is well developed in the calf. No evidence was found for the origin of true thyroid parenchyma from any part of the complex. The histological differentiation of the complex gave no indication of its value as an endocrine gland of any character, past or present.—Author's Abst., Wistar Bib. Service.

The recognition of myxedema. Kitchen, H. D., Proc. Central Soc. for Clinical Research, J.A.M.A. **104:** 149. 1935.

The term myxedema implies a profound metabolic disturbance and conjures up a picture of an individual whose mental and physical processes are greatly slowed, with expressionless face, trophic changes evidenced by thin dry hair and coarse dry nails, and exhibiting adiposity and a very marked intolerance to cold. One expects to find on examination a low blood pressure, slow pulse and a basal metabolic rate of minus 30 or lower. Women may give a history of sterility or amenorrhea. Such is the picture that most physicians visualize when thinking of a case of myxedema, and yet in spite of this definite clinical conception or perhaps because of it, many cases are overlooked. Diagnostically most practitioners are prone to be concerned primarily with the patient's most outstanding complaint and neglect often to see the patient as a whole. There are several reasons for this. In the first place the history may be misleading and the patient may not complain of undue fatigue because he may not remember feeling any different and may regard his condition as normal. Intolerance to cold may also be taken for granted and the wearing of additional clothing (such as a sweater) in a comfortably heated room or extra clothing when outdoors and the use of extra bedding may have become so fixed a part of the routine that it does not cause any voluntary mention by the patient. A careful history, however, will usually elicit these facts, which are of the greatest importance. Undue reliance on the basal metabolic rate and not enough on clinical evidence may prove fallacious, and

there is unfortunately a tendency to demand that, in a case in which the suspicions are aroused, the basal rate must of necessity conform to the low level usually expected before the diagnosis of myxedema can be made. The greatest cause for error is undoubtedly the focusing of the attention on symptoms. These may be very pronounced and at times very misleading, because they do not suggest the underlying endocrine disorder. Anemia, joint pains, flatulent dyspepsia, menorrhagia, extreme dyspnea, and chest pain of anginal character are some of the common symptoms of myxedematous patients. This presentation emphasizes some of the difficulties that result from undue concentration on symptoms. Diagnostic errors of this sort can be minimized if patients are regarded not as stomachs, hearts or chests but as individuals.

Results of fourth series of measurements of connection between radioactivity and goiter (Ergebnisse einer vierten Messungsserie zur Frage des Zusammenhangs zwischen Radioaktivität und Kropf). Lang, T., Ztschr. f. d. ges. Neurol. u. Psychiat. **152:** 107. 1935. Abst., J.A.M.A. **104:** 1375.

Lang employed the Elster-Deitel wire activation method in order to determine the amount of products of radioactive disintegration in the atmosphere, particularly in the lower layers, in order to be able to estimate the amount of radioactive gases (radon, thoron and actinon). As the amounts of these gases are influenced by the ground, he made tests in regions with certain geologicpetrographic characteristics. He found again, as he had in previous tests, that the atmosphere has the highest values of radioactivity in regions in which goiter is endemic. The values are lower in regions in which goiter is less frequent, and regions that are free from endemic goiter have the lowest values.

The insulin-sugar treatment of experimental pituitary hyperthyroidism. (Die Insulin-Zuckerbehandlung bei experimenteller hypophysärer Hyperthyreose). Loeser, A., Klin. Wchnschr. **12:** 83. 1934.

The injection of thyroid-stimulating substances of the anterior lobe of the pituitary causes a high degree of hyperfunction of the thyroid in young guinea-pigs. The stepping up of the efficiency of the thyroid and the increased secretion of the thyroid hormone in the blood give the symptoms of hyperthyroidism. The glycogen deficiency of the liver caused by the increased activity of the thyroid reaches its maximum on the 11th injection day. At that time the liver is almost glycogen-free. The glycogen loss of the liver cannot be compensated through simultaneous administration of high doses of carbohydrate (levulose). Insulin and sugar treatment of hyperthyroid animals invariably leads to an accumulation of glycogen reserves in the liver. The intensity of morphological changes in the thyroid is not influenced by the pancreas hormone.—G. L. Banay.

Dissociation of the thyroid from the sympathetic nervous system and reduction of the blood supply to the thyroid in angina pectoris. Lyon, J. A., and E. Horgan, South. M. J. **27:** 985. 1934.

Two cases of angina pectoris, in one of which there was accompanying congestive heart failure, are reported. The superior and inferior thyroid arteries were ligated thus denervating and lessening the blood supply to the thyroid. Both patients were relieved of anginal attacks and the one of his congestive failure; both were enabled to lead relatively active lives. At the time of publication the operation had been successfully applied in 3 additional cases of angina pectoris with relief of the anginal attacks.—Author's Abst. (Abbreviated).

Relationship between the function and iodine content of the thyroid glands (Beziehungen zwischen Funktion und Jodgehalt der Schilddrüse). Mansfeld, G., L. Scheffer and F. von Tyukody, Arch. f. exper. Path. u. Pharmakol. **176:** 344. 1934. Abst., Chem. Absts. **29:** 1148.

The iodine concentration of the 2 lobes of the thyroid gland (referred to dry weight) is the same in dogs. After the removal of one lobe, there is a considerable storage of iodine in the remaining lobe. This iodine accumulation is increased by the administration of iodine, but to the same degree whether the dog receives 20 or 100,000 gamma daily, and apparently depends upon the glandular activity rather than the amount of iodine intake. If the compensatory hypertrophy is diminished by the peroral administration of thyroid substance, there is only a slight increase in the thyroid iodine. Similarly, there is no post-operative storage in fasting animals,

even if they are given 100 gamma iodine daily. If the glandular activity is stimulated by the administration of thyrotropic hormone, iodine is promptly stored.

Physiology and principal interrelations of the thyroid. Marine, D., J.A.M.A. **104:** 2250. 1935.

The author states that the greatest advances in thyroid physiology during the last decade have been made in the chemistry of thyroxine and in the interrelations of the thyroid with other organs of internal and of external secretion. Hyperplasia indicates hyperactivity but not necessarily hyperfunction. Myxedema and cretinoid states may occur in individuals and animals with typical hyperplasia. It is more accurate to consider all functional hyperplasia as indicating relative or absolute iodine deficiencies and colloid goiter as the recovered, resting and physiologically normal stage. In the hyperplastic state the thyroglobulin is in the cells and is iodine-poor, while in the colloid state the thyroglobulin is mainly in the follicles and is usually iodine-rich. The iodine store is lower in the early spring and higher in the late summer. So far as is known, the thyroid is the only organ capable of elaborating an iodine-containing hormone. Thyroxine appears to be only the active chemical group of the true hormone, which is probably iodothyroglobulin. The most characteristic physiologic effect of iodothyroglobulin or its active chemical group thryoxine is that it increases after a latent period of 12 hours or more, the oxidation in the body of proteins, fats and carbohydrates and that it also increases the excretion of certain minerals, notably calcium and magnesium. The lowering of the iodine store and the tendency to thyroid hypertrophy in many of the infectious diseases clearly indicate that the thyroid is an important indirect factor in resistance to infections. Diet notably affects both the structure and the chemistry of the thyroid. The thyroid secretion is necessary for the specific dynamic action of foods. In thyroidectomized rabbits it disappears completely after about 65 days and can be restored in such rabbits by feeding desiccated thyroid. The thyrotropic factor of the anterior pituitary stimulates thyroid transplants in any part of the body to approximately the same degree that the non-transplanted thyroid is stimulated. The thyrotropic factor stimulates surviving thyroid cells in vitro and acts directly on the gland cells. Specific or intact nerve connections are unnecessary, either for secretory or for excretory activity of the gland. The interrelationship of thyroid sex glands has been recognized for centuries. The response of the pituitary to thyroidectomy is not limited to stimulating an increased production of the thyrotropic hormone but there is an increase in the growth hormone and the gonadotropic factors as well. Thyroidectomy hastens thymus involution. The thyroid hormone promotes glycogenolysis and because of this action the hypoglycemic action of insulin is increased after thyroidectomy and decreased by thyroid feeding. Recent observations suggest a direct stimulation of the chromaffin system by thyroxine. The adrenal cortex (and the sex glands), acting through the anterior pituitary, normally exercise some regulatory or inhibitory control over thyroid functions; when this control is sufficiently depressed or withdrawn the activity of the thyroid is temporarily increased. Clinicians have frequently reported improved water elimination in certain forms of nephritis by the administration of desiccated thyroid and more recently of thyroxine, alone or combined with parathyroid extract.—Abst., A.M.A. Service.

Effect of iodine on growth and metabolism of thyroid tissue. McCarrison, R., and S. Sankaran, Indian J. M. Research **21:** 184. 1933.

Cover slip preparations were made of 18-day chick-embryo thyroid in a plasma culture medium. Iodine was added in amounts of 12.5, 25, 50 or 100 gamma per 100 cc. The addition of 12.5 gamma of iodine per 100 cc. of the culture-medium had little effect either on the rate of growth of the thyroid tissue or on the metabolism of the cells. The 25 gamma had a pronounced stimulating effect on the growth of the tissue. It also increased the rate of metabolism of the growing cells as judged by the rapidity with which liquefaction of the medium occurred. In 50 gamma amount the iodine increased the rate of metabolism but had only a slight stimulating effect on growth. Bromine in 100 gamma amount had approximately the same effect as 50 gamma iodine. The addition of 100 gamma of iodine per 100 cc. greatly enhanced the rate of metabolism and halved the duration of cell life (to 40 hours).—R. G. H.

Treatment of diseases of the thyroid. Means, J. H., M. Clin. North America **19:** 1377. 1936.

The brief contribution on the management of thyroid disorders includes 5 illustrative case histories. The author voices the hope of many students in this field that before long there

might arise an adequate nonsurgical method of curing thyrotoxicosis in the light of recently discovered pituitary thyrotropic hormones and antihormones. However, Means still finds the practical treatment of toxic goiter to be iodinization followed by subtotal thyroidectomy. In the treatment of myxedema substitution therapy with thyroid substance is still dependable. In the management of thyroid neoplasms unaccompanied by functional changes, surgical treatment is indicated in the presence of pressure symptoms, suspicion of malignancy and for cosmetic reasons. Occasionally irradiation should supplement surgery.—I. B.

Quantitative investigations on the reflex excitability of normal and hyperthyroid animals (Quantitative Untersuchungen über Reflexerregbarkeit normaler und hyperthyreotischer Tiere). Muller, E., Arch. f. exper. Path. u. Pharmakol. **180:** 288. 1936.

Mature guinea-pigs were used in this experiment to determine the acoustic reflex response of normal and hyperthyroid animals, with and without ether narcosis. Three animals were used and the method employed depended on the ocular movement and body twitch of the experimental animal in response to timed blasts from a Galton whistle under a uniform air-pressure of 15 mm. The pitch of the whistle evoking the maximum response in each animal was determined. The recovery period was recorded after fatigue occurred. The hyperthyroid animals received 0.2 mg. thyroxin per diem for 5 days, exopthalmos developing after the fifth day. In normal animals the response to 1 stimulus per second was lost after 3 hours 21 min.; recovery occurred in 35 sec. and the threshold response was given at 6 mm. Thyroxin, 0.2 mg., increased the period of response prior to fatigue by 8 min. and with increasing doses of thyroxin reached a total of 25 minutes longer than the period of response in the control animal. The recovery period in the thyroxin animals was reduced to as low as 11 sec. and the threshold was lowered to 3 mm. Using 2 stimuli per sec. fatigue appeared in 17 minutes in the normal, recovery required 15 seconds; thyroxin animals receiving 2 stimuli per sec. fatigued after 27 minutes and recovered in 4 sec.; 4 stimuli per second produced fatigue in the normal animal within 16 seconds, and the recovery period was 4 seconds. Thyroxin animals receiving 4 stimuli per second fatigued after 33 seconds and recovered within 1 second. Ether narcosis lowered the excitability of the reflex but did not change the ratio of difference between the normal and the hyperthyroid animals in this experiment.—R. Cleveland.

Thyroid hyperplasia and goiter in hens kept in cages without gravel (Hyperplasie thyroïdienne et goitres chez des poules maintenues en cage sans gravier). Oberling, C., and M. Guérin, Compt. rend. Soc. de biol. **121:** 947. 1936.

Hens kept in gravel-free cages 6-15 months frequently developed enlarged thyroid glands. This enlargement, sometimes of 100% in weight, did not necessarily parallel the bone changes which occurred when the birds were deprived of gravel and which suggested parathyroid derangement.—J. C. D.

Cellular structure and cellular activity. With contributions to dynamic cytology of kidney and thyroid gland. Okkels, H., Skandinav. Arch. f. Physiol. **69:** 97. 1934. Abst., Chem. Absts. **28:** 5866.

The kidney and the thyroid were selected for this study of dynamic cytology, and especially in the thyroid was it possible to correlate the distinct morphological changes with the degree of metabolic function. The thyroid was stimulated with anterior pituitary extract, and the kidney with theophylline. The immediate effect was a swelling of the gland cells. The behavior of the Golgi apparatus, however, differs in these 2 types of cells, for whereas in the kidney no variation appears attributable to the functional changes, this shows a distinct increase in the thyroid cells under all forms of stimulation. The increase in the Golgi apparatus proceeds parallel to the rise in the metabolic rate, and this apparatus may therefore be considered as an indicator of the gland's specific activity. Similarly, the mitochondria in both types of cells behave differently, only in the thyroid there is a moderate increase, accompanying the stimulation of activity by anterior hypophysis extracts.